The American Journal of Medicine



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The American Journal of Medicine

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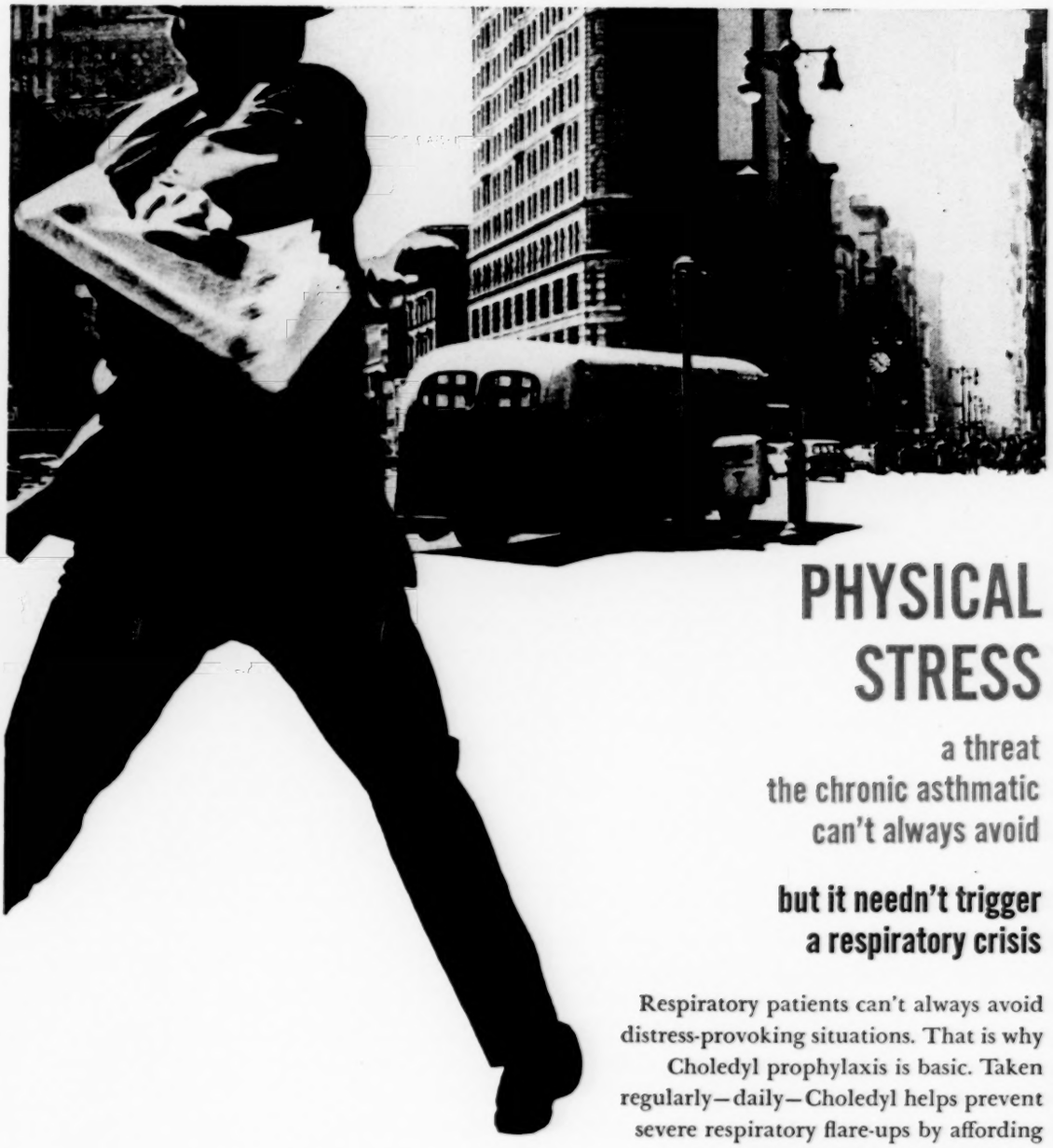
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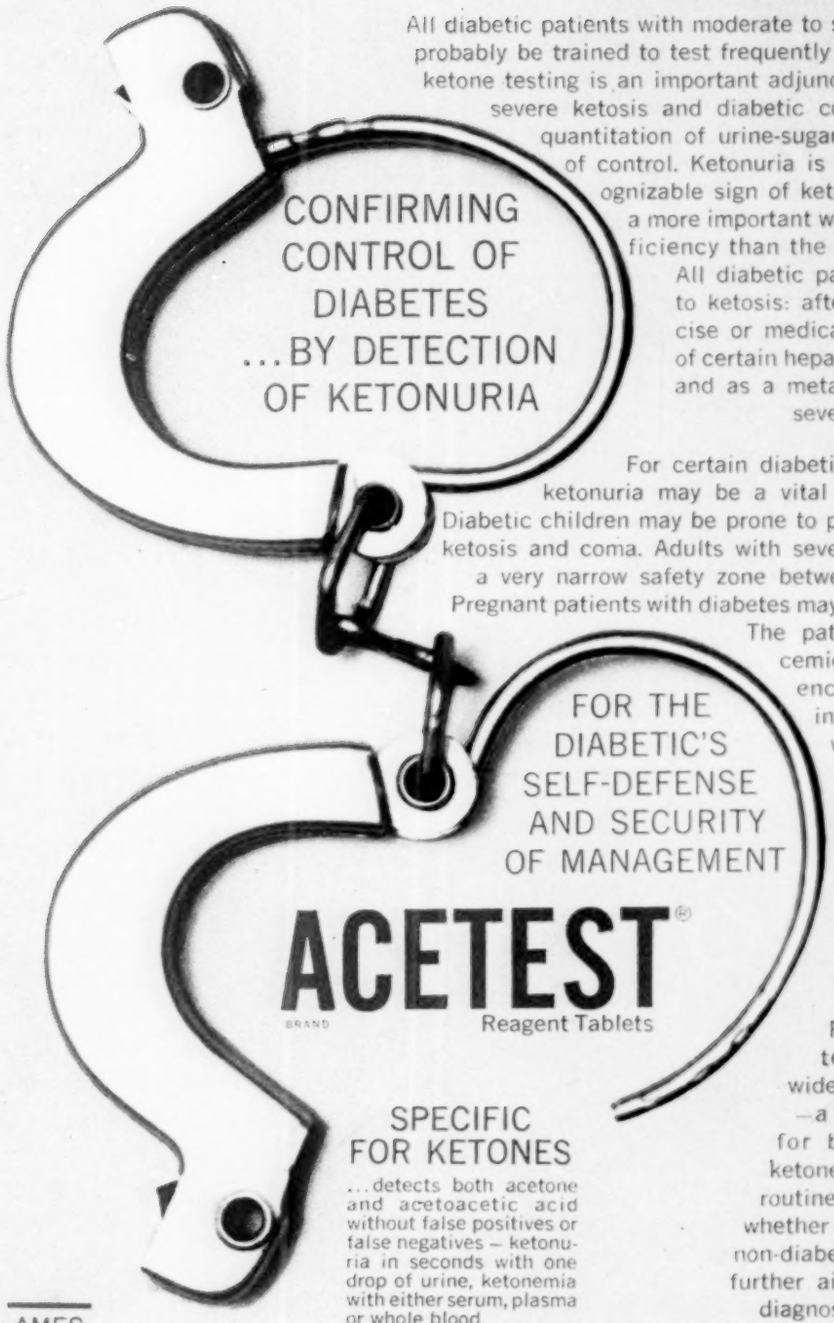
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(1) Duncan, G. G.: Diseases of Metabolism, ed. 4, Philadelphia, Saunders, 1959, p. 795.
(2) Lee C. T., and Duncan G. G.: Metabolism 5:144, 1956.

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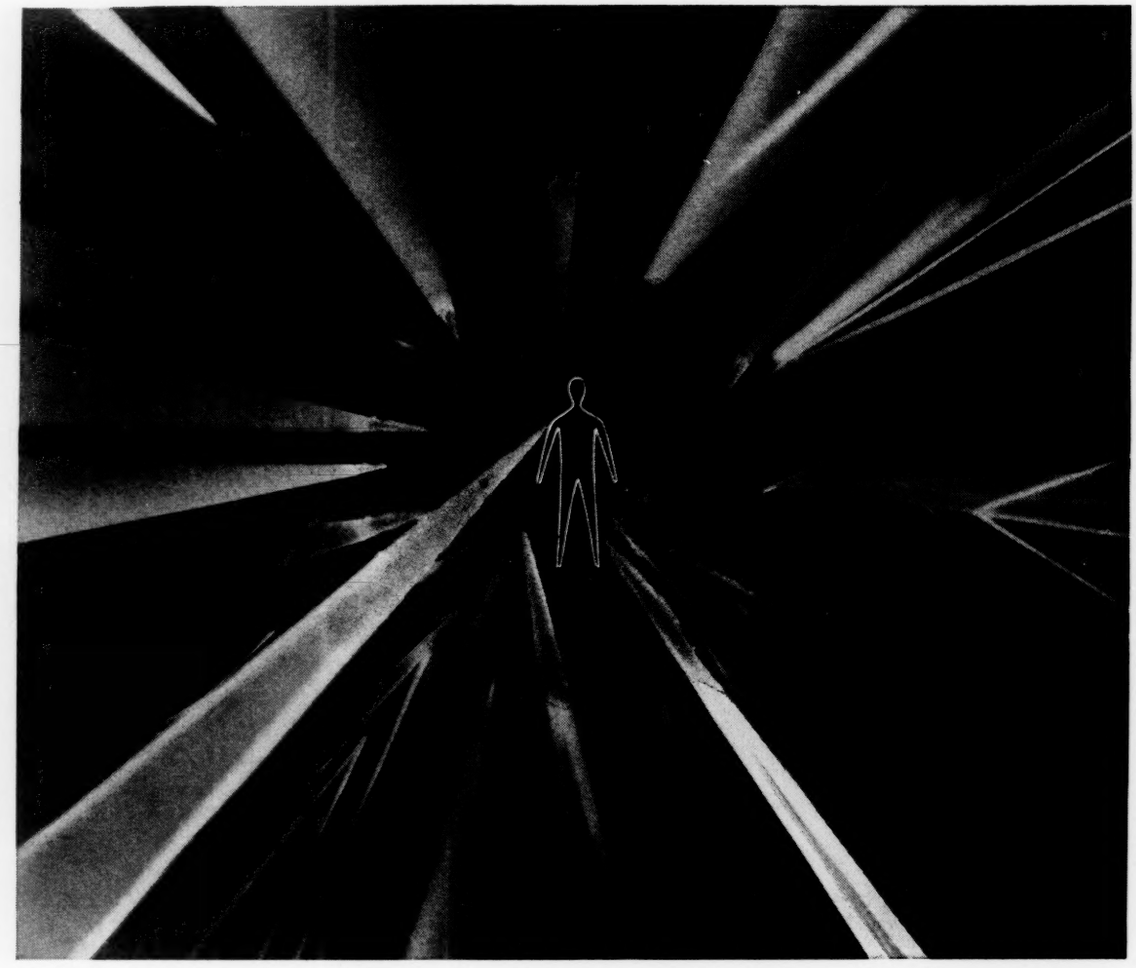
An interesting case.

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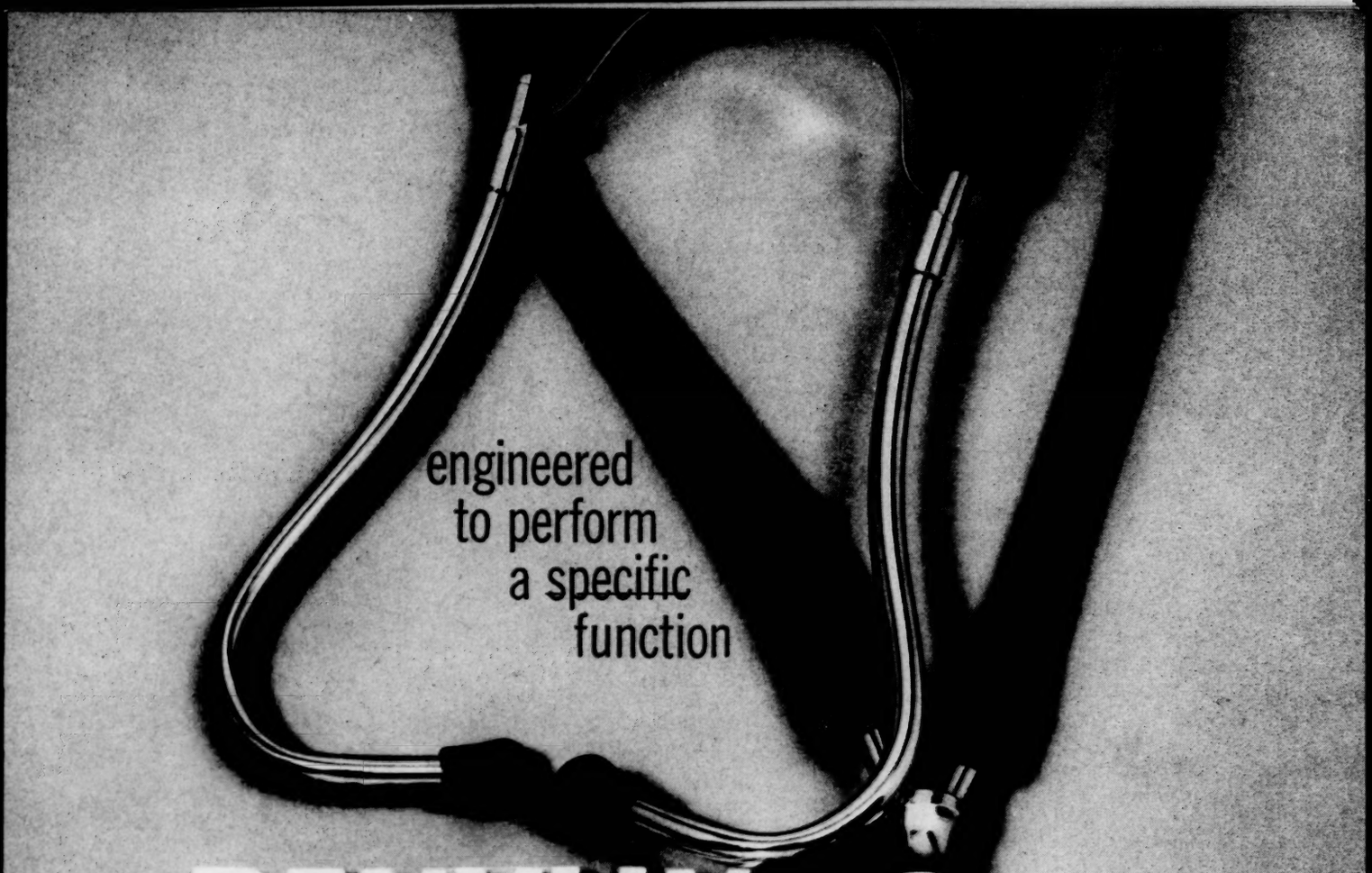
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1. Graham, W.: Canad. M. A. J. **82**:1005 (May 14) 1960. 2. Vaughn, P. P.; Howell, D. S., and Kiem, I. M.: Arth. and Rheumat. **2**:212, 1959. 3. O'Reilly, T. J.: J. Irish M. A. **46**:106, 1960. 4. Cardoe, N.: Ann. Rheumat. Dis. **18**:244, 1959. 5. Robichaux, E.: General Practice **24**:14, 1961. 6. Brooke, J. W.: Western Med. **2**:81, 1961. 7. Connell, J. F., Jr., and Rousselot, L. M.: Am. J. Surg. **98**:31, 1959. 8. Brodie, B. B., et al., in Contemporary Rheumatology 1956, p. 600. 9. Stein, I. D.: Ann. N. Y. Acad. Sc. **86**:307 (March 30) 1960. 10. Barczyk, W., and Röth, W.: Praxis **49**:589, 1960. 11. Miller, J. M., et al.: Antibiotic Med. and Clin. Therap. **7**:109, 1960. 12. Connell, J. F., Jr., and Rousselot, L. M.: Am. J. Surg. **97**:429, 1959. 13. Summary of individual case histories submitted to Geigy. 14. Domenjoz, R.: Ann. N. Y. Acad. Sc. **86**:263, 1960. 15. Smyth, C. J.: Ann. N. Y. Acad. Sc. **86**:292, 1960. 16. Yü, T. F., et al.: J. Pharmacol. and Exper. Therap. **123**:63, 1958.



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No. 4 of a series reporting on Endo Laboratories' Anticoagulant Survey

Nationwide Survey Explores Current Use of Anticoagulants in Venous Thrombosis and Arterial Embolism

Immediate institution of anticoagulant therapy is now accepted by most physicians for the control of thrombotic disease affecting the veins. By inhibiting further propagation of an already formed thrombus, anticoagulation helps to reduce disability and prevent new and potentially fatal thromboembolic episodes.

These concepts of treatment are supported by the responses of 10,016 physicians who contributed their experience to Endo Laboratories' *Anticoagulant Survey* completed earlier this year. Analysis of the data showed that the use of anticoagulation was more often therapeutic than prophylactic.

Indication	All Anticoagulants	
	Therapeutically	Prophylactically
Acute Thrombophlebitis	[illegible]	18.5%
Recurrent Chronic Thrombophlebitis	[illegible]	30.4%
Deep Venous Thrombosis	[illegible]	21.2%
Phlebothrombosis	[illegible]	20.0%
Arterial Embolism	[illegible]	22.5%
Pulmonary Embolism	[illegible]	25.1%

Figures refer to percentage of physicians prescribing oral anticoagulants

Specialists Lead in Therapeutic Application of Anticoagulants

The chart below indicates the use of Coumadin—the most widely prescribed oral anticoagulant among both general practitioners and specialists—in the foregoing conditions. Proportionately, anticoagulation with this agent was employed to a greater extent by internists and cardiologists than by the responding general practitioners. For example, 76.9% of 2,626 specialists prescribing Coumadin most often used the drug therapeutically in *deep venous thrombosis* with its associated danger of pulmonary embolism, compared to 56.2% of 3,092 general practitioners using Coumadin.

Indication	General Practitioners	Specialists
Acute Thrombophlebitis	[illegible]	82.5%
Recurrent Chronic Thrombophlebitis	[illegible]	67.9%
Deep Venous Thrombosis	[illegible]	76.9%
Phlebothrombosis	[illegible]	66.9%
Arterial Embolism	[illegible]	75.3%
Pulmonary Embolism	[illegible]	84.2%

Figures refer to percentage of physicians prescribing Coumadin® therapeutically

Although anticoagulants were used less often for prophylaxis than for therapy, it is noteworthy that in *recurrent chronic thrombophlebitis*, for example, as high as 38.2% of the reporting specialists and 26.5% of the general practitioners employed Coumadin prophylactically.

Anticoagulants Minimize Mortality Due to Pulmonary Embolism

Through the use of anticoagulants in venous thrombosis, mortality from subsequent pulmonary emboli "can be reduced from 18 per cent to less than 1 per cent."[1] Anticoagulation is an established measure of choice in the management of thrombophlebitis.[1-3] Mead and Wright[1] suggest that ligation be reserved for those cases in which anticoagulation fails to stop the thrombophlebitic process, since the late effects of ligation are often undesirable.

Selection of Coumadin as the oral anticoagulant of choice offers the advantages of rapid, consistent effect and "predictability of dosage."[3] For routine postoperative protection against pulmonary embolism, Coumadin "appears to be the most predictable and consequently the safest and most effective anticoagulant drug..."[4] Since Coumadin, the first clinically established warfarin sodium, is presented in parenteral dosage forms for I.V. or I.M. administration as well as in a broad range of tablet potencies, it is the most versatile anticoagulant in hospital and office practice.

1. Mead, A. W., and Wright, I. S.: M. Clin. North America 45:907, 1961. 2. Olwin, J. H., and Koppel, J. L.: S. Clin. North America 39:193, 1959. 3. Kirtland, H. B., Jr., et al.: California Med. 92:409, 1960. 4. Belding, H. H.: West. J. Surg. 68:84, 1960.

Coumadin (warfarin sodium) is manufactured under license from the Wisconsin Alumni Research Foundation, and is supplied as scored tablets of 2 mg., lavender; 2½ mg., orange; 5 mg., peach; 7½ mg., yellow; 10 mg., white; and 25 mg., red, as well as in 50 mg. and 75 mg. single-injection units.

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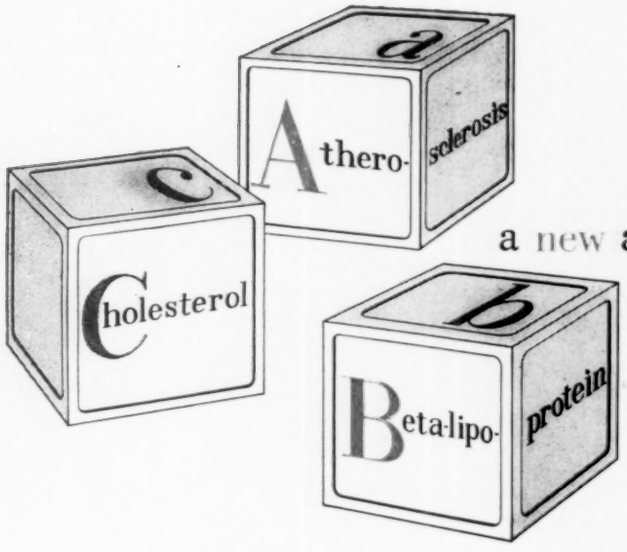
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the proven anticoagulant for long-term maintenance

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Endo® *ENDO LABORATORIES*
Richmond Hill 18, New York

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a new approach to these A-B-C's

A recent review of the atherosclerosis problem[1] points out that "cholesterol is only one of the serum lipids which may be related in some way to atherogenesis" and suggests that "specific triglycerides, phospholipids, fatty acids or the whole spectrum of beta-lipoproteins may be of equal or greater importance."

The role of the low-density beta-lipoproteins has been stressed by Olson[2], who suggests that they are the primary agents in atherosclerosis. Another recent discussion[3] has brought out the need for a simple and reliable testing procedure for beta-lipoproteins.

BETA-L TEST® is a specific immunochemical test for estimating serum beta-lipoprotein levels. BETA-L TEST reagent contains antibodies which specifically precipitate the beta-lipoproteins in human sera. This precipitate is then measured visually. Test requires only one drop of patient's serum and can be performed in 10 minutes. Clinical laboratories equipped with a micro-hematocrit centrifuge need no other special equipment for test performance.

In addition to its usefulness in atherosclerotic studies, BETA-L TEST may be found a helpful diagnostic and prognostic procedure in diabetes, hypothyroidism, the menopausal state and other conditions associated with elevated beta-lipoproteins.

BETA-L TEST measurements correlate well with total beta (low-density) lipoprotein measurements obtained by electrophoresis[4] and polyanion precipitation[4] and with cholesterol values in the beta-lipoproteins[5].

BETA-L TEST is supplied in 60-test kits.

1. Goldsmith, G. A.: Highlights on the Cholesterol-Fats, Diets and Atherosclerosis Problem, J.A.M.A. *176:* 783-790 (June 3) 1961.

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HYLAND LABORATORIES

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Hyland

Terramycin®

OXYTETRACYCLINE WITH GLUCOSAMINE

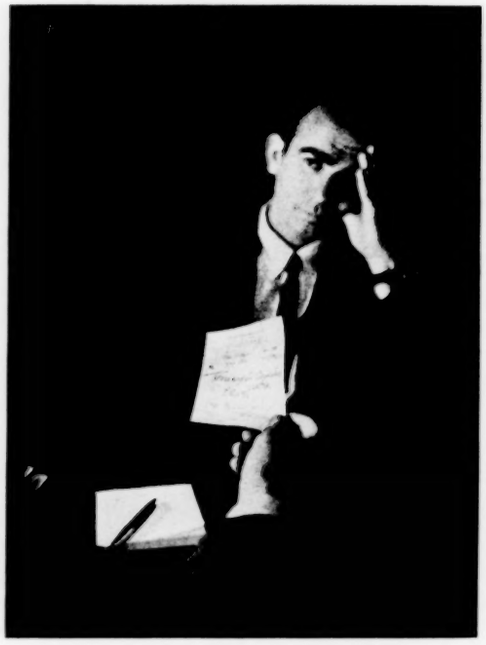
in sinusitis



According to a recent report* on the effectiveness of Terramycin in 106 cases of upper respiratory tract infection: "The response in sinusitis was particularly gratifying, as both acute and chronic cases were controlled within an average of five days."

"It was the impression of the hospital staff that oxytetracycline [Terramycin] was not only better tolerated, but more effective than other antibiotics habitually used."

The results reported in this and many other studies confirm the vitality of Terramycin for broad-spectrum antibiotic therapy and demonstrate why —increasingly—the trend is to Terramycin.



In brief

The dependability of Terramycin in daily practice is based on its broad range of antimicrobial effectiveness, excellent toleration, and low order of toxicity. As with other broad-spectrum antibiotics, overgrowth of nonsusceptible organisms may develop. If this occurs, discontinue the medication and institute appropriate specific therapy as indicated by susceptibility testing. Glossitis and allergic reactions to Terramycin are rare. Aluminum hydroxide gel may decrease antibiotic absorption and is contraindicated.

More detailed professional information available on request.

another reason why the trend is to Terramycin—*versatility of dosage form:*

TERRAMYCIN Syrup/Pediatric Drops
125 mg. per tsp. and 5 mg. per drop (100 mg./cc.), respectively—deliciously fruit-flavored aqueous forms . . . preconstituted for ready oral administration

TERRAMYCIN Intramuscular Solution
50 mg./cc. in 10 cc. vials; 100 mg. and 250 mg. in 2 cc. ampules—the broad-spectrum antibiotic for immediate intramuscular injection . . . conveniently preconstituted . . . notably well tolerated at injection site with low tissue reaction compared to other broad-spectrum antibiotics

Terramycin®

OXYTETRACYCLINE WITH GLUCOSAMINE

CAPSULES *250 mg. and 125 mg. per capsule*

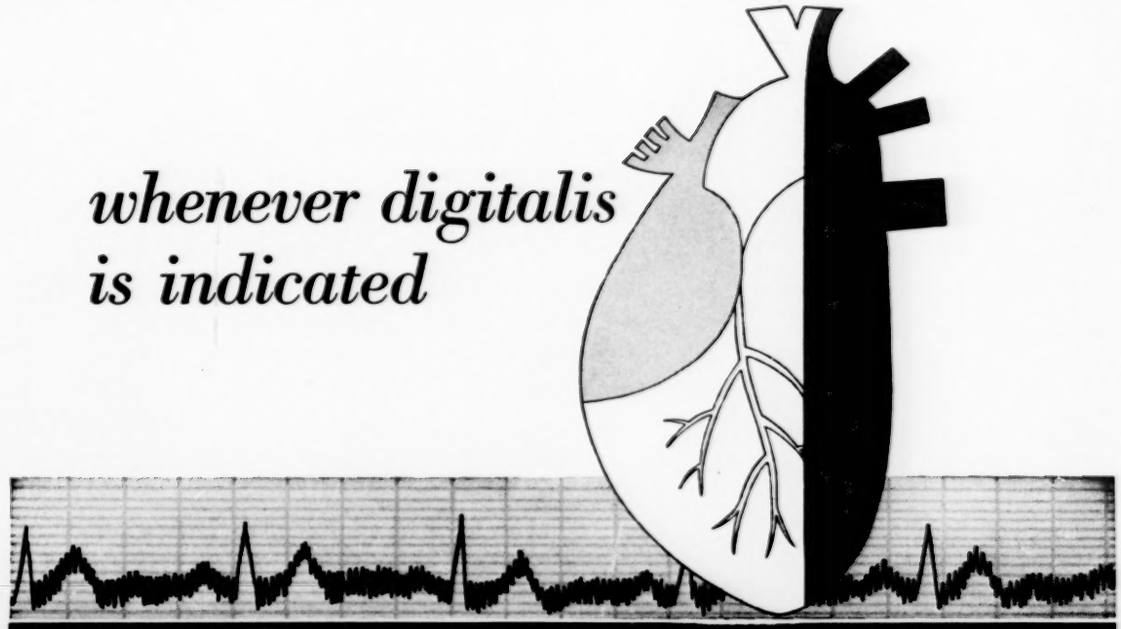
convenient initial or maintenance therapy in adults and older children

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*Jacques, A. A., and Fuchs, V. H.: J. Louisiana M. Soc. 113:200, May, 1961.

whenever digitalis is indicated



'LANOXIN'® brand DIGOXIN

formerly known as Digoxin 'B. W. & Co.'

"If one digitalis agent were to be recommended for its adaptability to the many and varied clinical contingencies, we believe Digoxin would be the drug of choice."

Lown, B., and Levine, S. A.: Current Concepts in Digitalis Therapy, Boston, Little, Brown & Company, 1954, p. 23, par. 2.

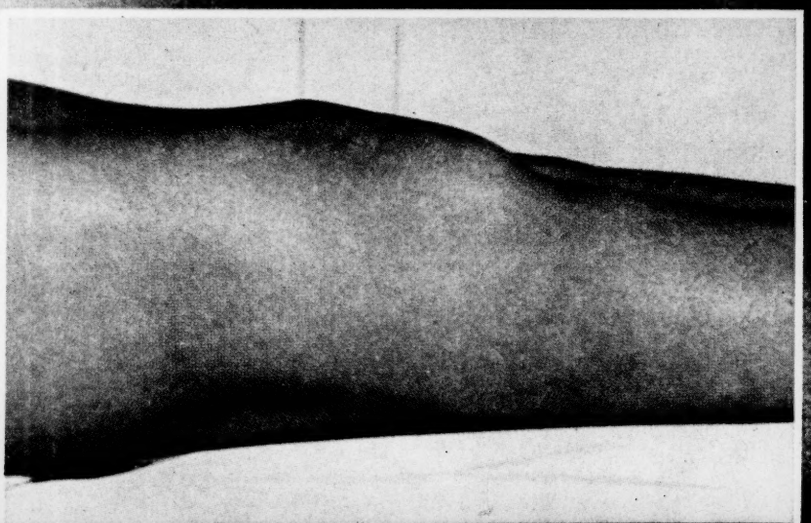
'LANOXIN' TABLETS	'LANOXIN' INJECTION	'LANOXIN' ELIXIR PEDIATRIC
0.25 mg. scored (white) 0.5 mg. scored (green)	0.5 mg. in 2 cc. (I.M. or I.V.)	0.05 mg. in 1 cc.

BURROUGHS WELLCOME & CO. (U.S.A.) INC., Tuckahoe, N.Y.



Female, Age 45. Diagnosis: Rheumatoid Arthritis, Class III, Stage III. Onset of disease was approximately 10 years ago. During this period, patient has never been asymptomatic. Various courses of cortisone therapy afforded partial relief. Immediately prior to institution of therapy with CELESTONE there was swelling and or tenderness over both sternoclavicular joints, in shoulders, wrists, ankles, feet and the fingers which also had spindle-shaped deformities; limitation of motion in both knees. Considerable swelling with synovitis observed in right knee; patient unable to extend it fully when lying flat. Physical examination also revealed patient to be badly undernourished; there were no other significant findings. Rx: CELESTONE Tablets, 0.6 mg., q.i.d. Photo 1 shows patient prior to therapy with CELESTONE.

Arthritic/inflammatory flare-up



Results: Within 48 hours, both subjective and objective improvement noted. Subsidence of pain began, and swelling substantially decreased. After 7 days, patient was completely asymptomatic and able to function normally in her environment. Dosage gradually reduced by weekly decrements of 0.3 mg. to a maintenance level of 0.3 mg. daily. Patient has remained asymptomatic, has gained 12 pounds and has returned to her normal weight. No side effects reported. Photo 2 shows patient after 7 days' therapy with CELESTONE. Note 50 per cent decrease in knee size.

Photographs courtesy of Abraham Cohen, M.D., Philadelphia, Pa.

Schering

An important new agent for steroid therapy: Twenty months of pre-introductory clinical trials have demonstrated that CELESTONE provides unexcelled antiarthritic and anti-inflammatory effects with significantly lower milligram dosages than those required with most other steroids. These studies have also established its "low incidence of side effects... [and] absence of new toxic effects..."[1]

Unsurpassed effectiveness in rheumatoid arthritis: In a series of 37 patients previously treated with other corticosteroids, CELESTONE was observed to produce an enhanced antiarthritic effect in over 50 per cent of the cases: "Better over-all improvement, as reflected in greater relief from pain, decreased inflammation, increased range of motion and constitutional benefits, was reported by the majority of patients in this series."[2]

In another group of patients studied, 88.8 per cent of whom were much improved or improved on CELESTONE, the authors noted that "results were not affected by either the class or the stage of rheumatoid arthritis; in fact, all but two of our Class III and Stage III patients obtained maximal improvement with betamethasone [CELESTONE]."[3]

Rapid remission with Celestone

Gratifying results have been achieved with CELESTONE in a broad range of steroid-responsive disorders, from rheumatoid arthritis to bronchial asthma, allergic dermatoses, and inflammatory ocular diseases. Rapid subsidence of arthritic flare-up can usually be expected on average daily dosages of from 2 to 4 tablets. The single tablet strength (0.6 mg.) facilitates dosage schedules and proper adjustment when patients are switched from other corticosteroids.

CELESTONE "appears to satisfy the criteria for an improved corticosteroid in rheumatoid arthritis. It exerts its antirheumatic and anti-inflammatory activity at lower dosages than other steroids available for the management of this disease... our data indicate that therapy with this steroid is attended by a substantially lower incidence of untoward effects... [and] has not been shown to cause any new side effects..."[3]

For complete details, consult latest Schering literature available from your Schering Representative or the Medical Services Department, Schering Corporation, Bloomfield, New Jersey.

Cited References: 1. Frank, L.: The Place of Betamethasone in Dermatologic Practice, Paper presented at First Conference on the Clinical Application of Betamethasone—A New Corticosteroid, New York City, May 8, 1961. 2. Kammerer, W.H.: Observations on the Effects of Betamethasone in Rheumatoid Arthritis. *Ibid.* 3. Cohen, A., and Goldman, J.: Management of Rheumatoid Arthritis with a New Steroid. *Ibid.* **Additional References:** 4. Nierman, M.M.: The Use of Betamethasone in Dermatology. *Ibid.* 5. Gant, J.Q., Jr., and Gould, A.H.: Betamethasone: A Clinical Study. *Ibid.* 6. Dresner, E., and Cathcart, E.S.: The Anti-Inflammatory Activity of Betamethasone, A New Glucocorticoid Epimer. *Ibid.* 7. Cecil, R.L.: Continued Progress in Corticosteroids. *Ibid.* 8. Bedell, H.: A New Systemic Steroid in the Treatment of Allergies in Office Practice. *Ibid.* 9. Goldman, L.: Investigation of a New Steroid in Dermatology. *Ibid.* 10. Hampton, S.F.: Betamethasone—A New Steroid in Allergy: A Preliminary Report. *Ibid.* 11. Bukantz, S.C.: Observations on the Use of Betamethasone in the Intractable Asthmatic Child. *Ibid.* 12. Schwartz, E.: Clinical Evaluation of Betamethasone in Chronic Intractable Bronchial Asthma. *Ibid.* 13. Gordon, D.M.: Betamethasone—A New Corticosteroid in Ophthalmology. *Ibid.* 14. Abrahamson, I.A., Jr.: A Clinical Evaluation of Betamethasone. *Ibid.*

H-397

(brand of betamethasone) **Tablets, 0.6 mg.**

CELESTONE®

a corticosteroid advance from Schering

Announcing CAPLA™ – to lower blood pressure

Only Capla acts directly on the brainstem centers that control blood pressure

This central action reduces peripheral resistance, lowers pressure.

Capla is a new kind of drug to treat hypertension. Chemically, Capla is 2-methyl-2-*sec*-butyl-1, 3-propanediol dicarbamate. It is unrelated chemically to any other antihypertensive agent.

New therapy

Capla is effective alone in the treatment of mild to moderate hypertension, and can be combined with diuretics or peripherally acting antihypertensives in more severe cases.

Exceptionally well tolerated

Capla acts rapidly, producing substantial blood pressure reduction within two hours, yet does not produce postural hypotension. It has proved exceptionally well tolerated in clinical use and has no known contraindications. Capla has not produced changes in renal, hematological, hepatic or endocrine function.

Compatible with other drugs

Hypertensive patients with other disorders can receive Capla along with other medications.

For example, patients with congestive heart failure, angina, and diabetes mellitus can receive Capla along with such medications as digitalis, nitrates, and insulin—without aggravating these other disorders.

a new drug that works in a new way without serious side effects

Capla lowers blood pressure without serious side effects

In 755 hypertensive patients treated with Capla, the only side effect observed with any frequency was mild, usually transient, drowsiness.

CAPLA™

CENTRAL ACTING PRESSURE LOWERING AGENT

Wallace Laboratories Cranbury, New Jersey

Literature and samples to physicians on request

Capla is effective therapy without the unpleasant and serious side effects which often cause patients to abandon treatment. Side effects, when they do occur, are mild and usually transient. Transient drowsiness sometimes occurs, usually at higher dosage.

Patients on Capla often report a mild calming effect which, together with Capla's unusual freedom from side effects, makes therapy gratifying for both patient and physician.

Effective in clinical use

Average reductions in systolic and diastolic blood pressure reported with Capla. (755 patients)

	BEFORE	AFTER
MILD (403 patients) (Diastolic B.P. up to 100 mm. Hg)	183/93	159/85
MODERATE (249 patients) (Diastolic B.P. from 101 to 115 mm. Hg)	187/108	169/94
SEVERE (103 patients) (Diastolic B.P. over 116 mm. Hg)	208/120	162/99

Usual dose, Capla 300 mg., q.i.d.—duration of therapy, 3 weeks to over 1 year. These data show Capla reduces both systolic and diastolic pressure, usually in proportion to initial pre-treatment elevations.

Recommended Dosage: One 300 mg. tablet three or four times daily, before meals and at bedtime. Dosage should be adjusted to individual requirements.
Composition: Each white, scored tablet is 300 mg. of Capla (mebutamate, Wallace).
Supplied: Bottles of 100 tablets.

Clinical and Pharmacological Reports 1. Berger, F.M., Douglas, J.F., Kletzkin, M., Ludwig, B.J., and Margolin, S.: The Pharmacological Properties of 2-methyl-2-sec-butyl-1, 3-propanediol dicarbamate (mebutamate, W-583) a New Centrally Acting Blood Pressure Lowering Agent. J. Pharmacology. In press, 1961. **2.** Berger, F.M., and Margolin, S.: A Centrally Acting Blood Pressure Lowering Agent (W-583). Fed. Proc. 20: 113 1961. **3.** Bohensky, F.S.: Mebutamate, a New Drug for the Treatment of Hypertension, N.Y. State J. Med. In press, 1961. **4.** Diamond, S. and Schwartz, M.: Blood Volume in Uncomplicated Essential Hypertension. Modern Med. In press, 1961. **5.** Douglas, J.F., Ludwig, B.J., Ginsberg, T. and Berger, F.M.: Studies on W-583 Metabolism, Fed. Proc. 20: 113 1961. **6.** Duarte, C., Brest, A.N., Kodama, R., Naso, F., and Moyer, J.H.: Observation on the Antihypertensive Effectiveness of a new Propanediol Dicarbamate (W-583). Curr. Therap. Res. 2: 148-52 May, 1960. **7.** DuChez, J.W., Scientific Exhibit, American Medical Association, New York, 1961. **8.** Hoffoman, J.L.S., Jr.: Treatment of Hypertensive Patients with Mebutamate, a new Antihypertensive drug—A Preliminary Report. J. Nat. Med. Assoc. In press, 1961. **9.** Kletzkin, M., and Berger, F.M.: A Centrally Acting Antipressor Agent. Fed. Proc. 20: 113 1961. **10.** Leslie, C.H.: The Efficacy of Mebutamate, A New Antihypertensive Drug, in the Treatment of Refractory Geriatric Hypertensive Patients—A Preliminary Report. J. Am. Geriatrics Soc. In press, 1961. **11.** Mulinos, M.G., Scientific Exhibit, Amer. College of Cardiology, New York, May, 1961. **12.** Mulinos, M.G., Salteiors, S., Boyd, L.J., and Cronk, G.A.: Human Pharmacology Studies with W-583. Fed. Proc. 20: 113 1961. **13.** Shubin, H., Scientific Exhibit, American College of Cardiology, New York, 1961. **14.** Turek, L.H.: Clinical Evaluation of Mebutamate, an Antihypertensive Agent: Preliminary Report. Clinical Med. 8: 7 1961.



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NEOMYCIN—actively bactericidal against a wide range of gram-negative intestinal pathogens, but relatively ineffective against certain diarrhea-causing organisms.

SULFASUXIDINE®, succinylsulfathiazole—an ideal adjunct to neomycin because it is highly effective against Clostridia and certain other neomycin-resistant organisms.

KAOLIN AND PECTIN—coat and soothe the inflamed mucosa, adsorb toxins, help provide rapid symptomatic relief.

Additional information on CREMOMYCIN is available to physicians on request.

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the first few minutes are decisive in aborting a full-blown migraine attack

sublingual Ergomar stops migraine attack with parenteral*speed

(PROVIDES RELIEF TWICE AS FAST AS ORAL FORMS)

In classic migraine, other migraines or any vascular headache, Ergomar combines the proven effectiveness of ergotamine tartrate with the unparalleled convenience and speed of sublingual administration. Unlike tablets, injectables or suppositories, Ergomar can be taken at home, or en route—anytime, anywhere. Within 30 seconds, plus only circulation time, cerebral effects begin building up—even faster than the time needed to obtain or prepare an injection.

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SAMPLES AND LITERATURE AVAILABLE ON REQUEST.

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Unquestioned therapeutically

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TRISEM

SUSPENSION (chocolate-flavored)

For the many bacterial infections that respond promptly to triple sulfonamide therapy, Trisem presents a preferred formula.

Each 5 cc. contains, in a pleasant, chocolate-flavored vehicle:

Sulfamerazine (microcrystalline) . . . . . 0.167 Gm. (2 3/5 gr.)
Sulfadiazine (microcrystalline) . . . . . 0.167 Gm. (2 3/5 gr.)
Sulfamethazine (microcrystalline) . . . . 0.167 Gm. (2 3/5 gr.)

Each 5 cc. (1 tsp.) provides 0.5 Gm. total sulfonamides.

Each 15 cc. (1 tbsp.) provides 1.5 Gm. total sulfonamides.

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- **unquestioned therapeutic value**
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- **broad antibacterial spectrum**
- **provides high blood levels promptly**
- **economical for the patient**

Many physicians prefer to use the sulfonamides to control bacterial infections because resistant organisms rarely develop. Nor, as with some broad spectrum therapy, is there the complication of such after-effects as moniliasis.

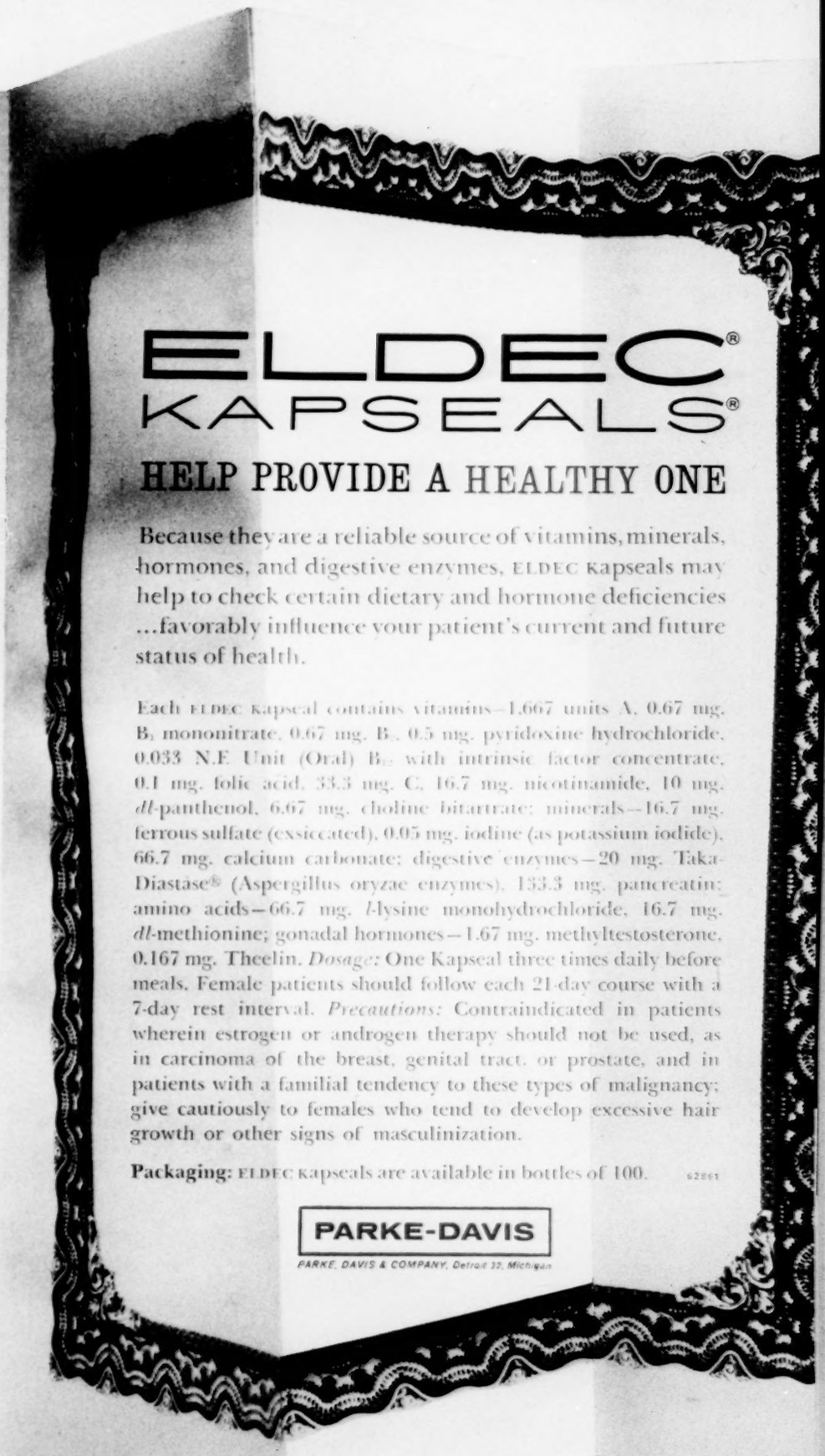
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Each ELDEC Kapseal contains vitamins—1,667 units A, 0.67 mg. B_1 mononitrate, 0.67 mg. B_6, 0.5 mg. pyridoxine hydrochloride, 0.033 N.F. Unit (Oral) B_{12} with intrinsic factor concentrate, 0.1 mg. folic acid, 33.3 mg. C, 16.7 mg. nicotinamide, 10 mg. *dl*-panthenol, 6.67 mg. choline bitartrate; minerals—16.7 mg. ferrous sulfate (exsiccated), 0.05 mg. iodine (as potassium iodide), 66.7 mg. calcium carbonate; digestive enzymes—20 mg. Taka-Diastase® (Aspergillus oryzae enzymes), 133.3 mg. pancreatin; amino acids—66.7 mg. *l*-lysine monohydrochloride, 16.7 mg. *dl*-methionine; gonadal hormones—1.67 mg. methyltestosterone, 0.167 mg. Theelin. *Dosage:* One Kapseal three times daily before meals. Female patients should follow each 21-day course with a 7-day rest interval. *Precautions:* Contraindicated in patients wherein estrogen or androgen therapy should not be used, as in carcinoma of the breast, genital tract, or prostate, and in patients with a familial tendency to these types of malignancy; give cautiously to females who tend to develop excessive hair growth or other signs of masculinization.

Packaging: ELDEC Kapseals are available in bottles of 100. 62861

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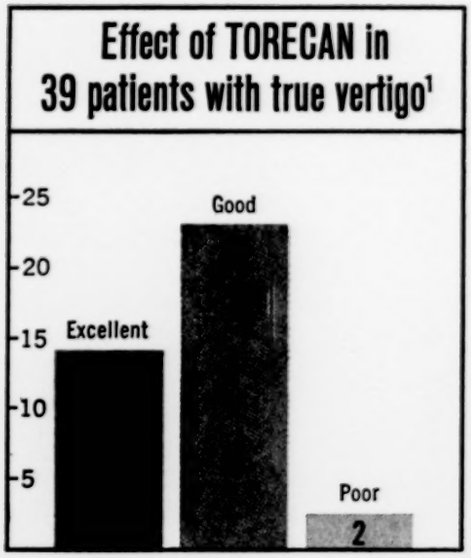
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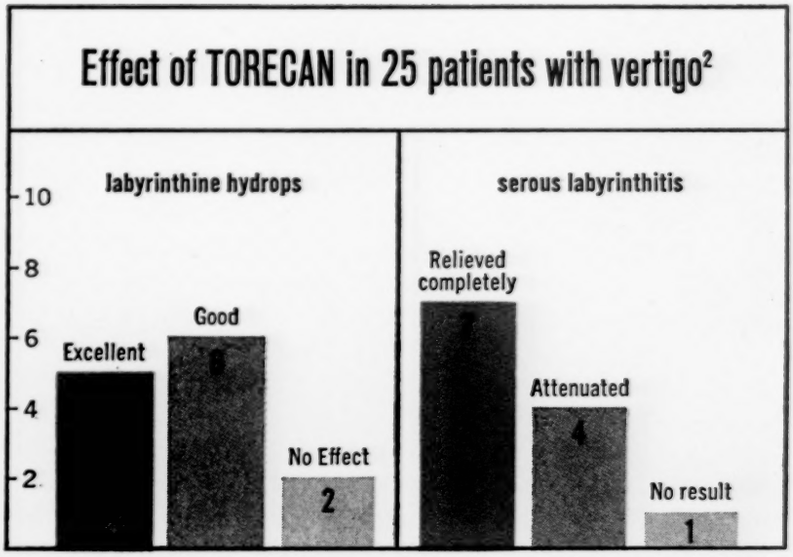

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"This study has shown Torecan to be an effective and well-tolerated preparation in the treatment of vertigo, nausea and vomiting associated with a variety of otolaryngologic disorders. The high degree of efficacy exhibited by Torecan in relieving vertigo was especially noteworthy."[2]

AVERAGE ADULT DOSAGE: Oral: 1 tablet (10 mg.) three times daily.
Intramuscular: 10-20 mg. daily.

SUPPLY: TABLETS, 10 mg.
AMPULS, 2 cc. (10 mg.).
Each cc. contains 5 mg. Thiethylperazine Maleate. Inactive ingredients: sodium metabisulfite 0.25 mg., sodium bicarbonate 1.0 mg., propylene glycol 50 mg., water for injection q.s. to 1.0 cc.

PRECAUTIONS: It is obvious that sound clinical judgment must be exercised in determining whether vomiting represents a warning of organic abnormality and that this must first be recognized before employing a potent antiemetic such as Torecan.

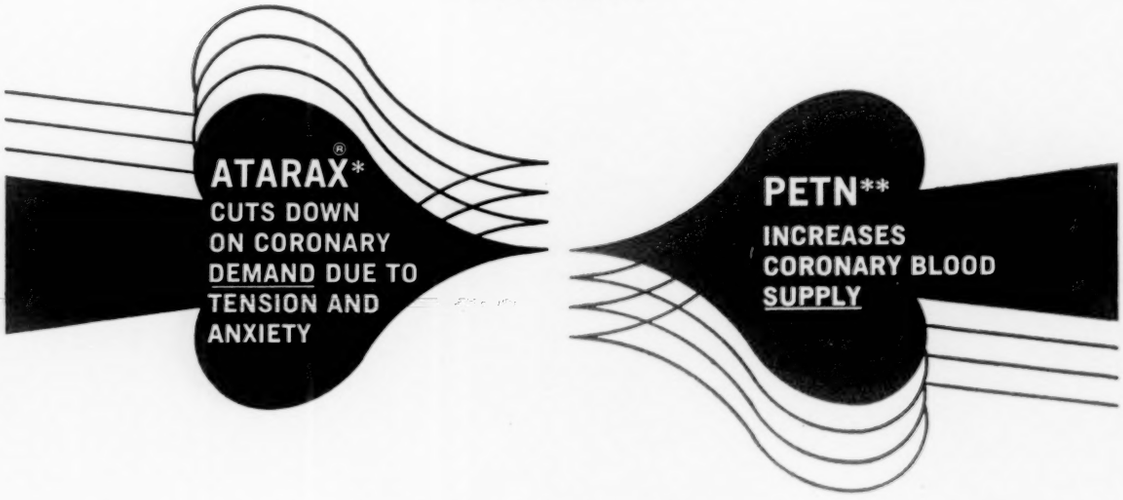
Drowsiness and/or dryness of the mouth may occur with doses above 30 mg. daily. While no hepatic, hematopoietic or renal toxicity have been reported at recommended dose levels, it should be remembered that these are reactions which may occur with the phenothiazine group. Orthostatic hypotension may be manifested at higher dose levels. Torecan is contraindicated in severely depressed or comatose states from any cause. In excessive doses, Torecan may produce extrapyramidal stimulation with the varied symptom complex characteristic of this complication. Ampuls are recommended for **intramuscular injection only.**

1. Kearby, N.: Thiethylperazine dimaleate in the treatment of vertigo, presented at Louisiana State Medical Society, New Orleans, May 10, 1961.
2. Rubin, W.: Clinical evaluation of Torecan in the treatment of vertigo, to be published.

SANDOZ

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they decrease "length, severity, and amount of angina pectoris" in anxious cardiacs.[1]

Give your angina patient better protection by balancing supply and demand...with CARTRAX.

note: Should be given with caution in glaucoma.

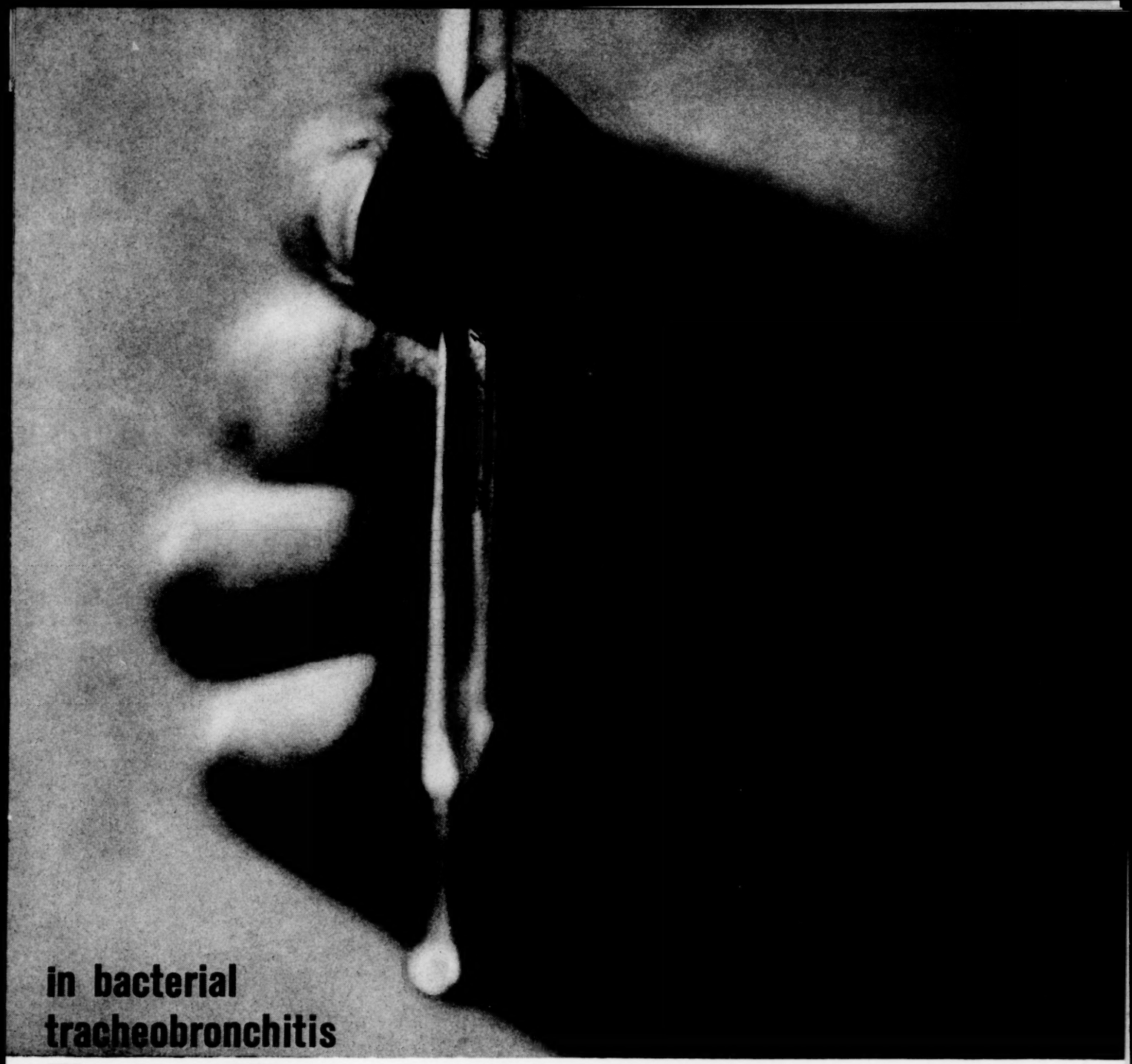
dosage: Begin with 1 to 2 yellow CARTRAX "10" tablets (10 mg. PETN plus 10 mg. Atarax) 3 to 4 times daily. When indicated, this may be increased by switching to pink CARTRAX "20" tablets (20 mg. PETN plus 10 mg. Atarax). For convenience, write "CARTRAX 10" or "CARTRAX 20."
Supplied in bottles of 100. Prescription only.

1. Clark, T. E., and Jochem, G. G.: Angiology **11**:361 (Aug.) 1960.

*brand of hydroxyzine **pentaerythritol tetranitrate

Roerig

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Science for the World's Well-Being®



in bacterial tracheobronchitis

Panalba* promptly

to gain precious therapeutic hours

Panalba  **your broad-spectrum antibiotic of first resort**

In the presence of bacterial infection, taking a culture to determine bacterial identity and sensitivity is desirable—but not always practical in terms of the time and facilities available.

A rational clinical alternative is to launch therapy at once with Panalba, the antibiotic that provides the best odds for success.

Panalba is effective (in vitro) against 30 common pathogens, including the ubiquitous staph. Use of Panalba *from the outset* (even pending laboratory results) can gain precious hours of effective antibiotic treatment.

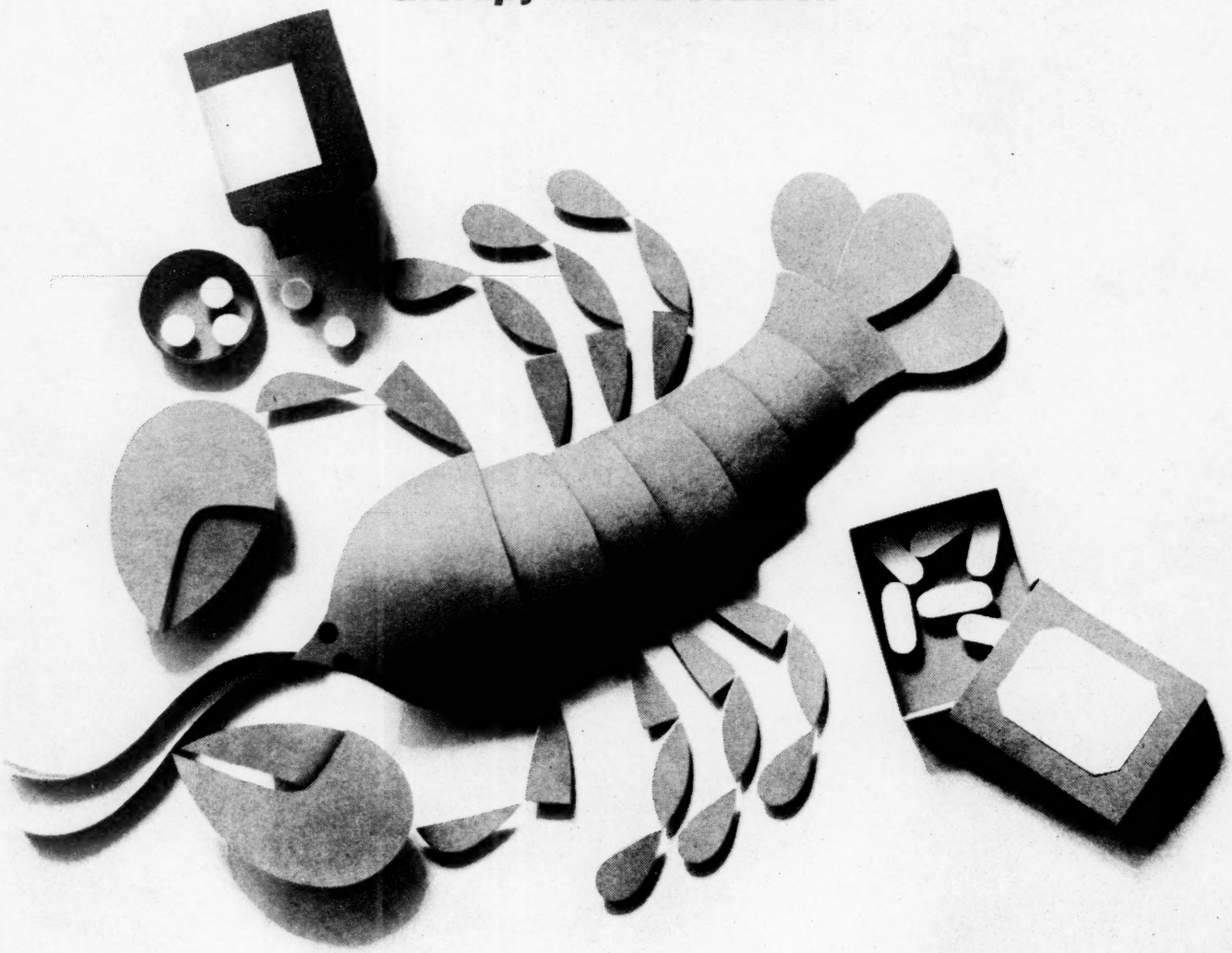
Supplied: Capsules, each containing Panmycin* Phosphate (tetracycline phosphate complex), equivalent to 250 mg. tetracycline hydrochloride, and 125 mg. Albamycin,* as novobiocin sodium, in bottles of 16 and 100.
Usual Adult Dosage: 1 or 2 capsules 3 or 4 times a day.
Side Effects: Panmycin Phosphate has a very low order of toxicity comparable to that of the other tetracyclines and is well tolerated clinically. Side reactions to therapeutic use in patients are infrequent and consist principally of mild nausea and abdominal cramps.
Albamycin also has a relatively low order of toxicity. In a certain few patients, a yellow pigment has been found in the plasma. This pigment, apparently, a metabolic by-product of the drug, is not necessarily associated with abnormal liver function tests or liver enlargement.
Urticaria and maculopapular dermatitis, a few cases of leukopenia and thrombocytopenia have been reported in patients treated with Albamycin. These side effects usually disappear upon discontinuance of the drug.
Caution: Since the use of any antibiotic may result in overgrowth of nonsusceptible organisms, constant observation of the patient is essential. If new infections appear during therapy, appropriate measures should be taken.
Total and differential blood counts should be made routinely during prolonged administration of Albamycin. The possibility of liver damage should be considered if a yellow pigment, a metabolic by-product of Albamycin, appears in the plasma. Panalba should be discontinued if allergic reactions that are not readily controlled by antihistaminic agents develop.

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in severe drug and food sensitivity... rapid relief and control of symptoms on short-term therapy with Decadron®



Brief treatment with DECADRON – orally or parenterally – can provide rapid and effective control of allergic emergencies and acute allergic disorders such as reactions to foods, drugs, plants, weeds, and animals. In 40 patients given Injection DECADRON Phosphate, "subjective improvement was often noticed within one hour and objective improvement recorded within 4 hours."[1] Therapeutic doses of steroids may help prevent recurrences of severe allergic states, without interfering with desensitization or other immunity procedures.[2]

Before prescribing or administering DECADRON, the physician should consult the detailed information on use accompanying the package or available on request.

References: 1. Grater, W.C.: Southern M. J. 53:1144, 1960. 2. Feinberg, S. M.: Med. Sci. 6:(No. 3) 181, 1959.

Supplied: As 0.75 mg. and 0.5 mg. scored, pentagon-shaped tablets in bottles of 100 and 1000. As Injection DECADRON Phosphate in 5 cc. vials, each cc. containing 4 mg. of dexamethasone 21-phosphate as the disodium salt; inactive ingredients: 8 mg. creatinine, 10 mg. sodium citrate; sodium hydroxide to pH 7.8, and water for injection q. s. 1 cc.; preservatives: 0.32 per cent sodium bisulfite and 0.5 per cent phenol. DECADRON is a trademark of Merck & Co., Inc.

MERCK SHARP & DOHME Division of Merck & Co., INC., West Point, Pa.

Decadron®

Dexamethasone

DECADRON: Recommended dosage schedule in the treatment of drug and food sensitivity reactions

time	amount	administration
1st day	one to two cc. (4 to 8 mg.) Injection DECADRON Phosphate intramuscular	repeated as necessary (In substituting tablet therapy, give the first oral dose four or five hours before the final parenteral dose.)
2nd day	two 0.75 mg. Tablets DECADRON	b.i.d.
3rd day	two 0.75 mg. Tablets DECADRON	b.i.d.
4th day	one 0.75 mg. Tablet DECADRON	b.i.d.
5th day	one 0.75 mg. Tablet DECADRON	per day
6th day	one 0.75 mg. Tablet DECADRON	per day
7th day	RETURN VISIT	



to prevent pain and anxiety in angina

For your angina patients, EQUANITRATE helps control pain and angina-triggering anxiety. EQUANITRATE reduces the number and severity of attacks, increases exercise tolerance, and lessens nitroglycerine dependence. Russek† reports "The best results . . . in both clinical and electrocardiographic response, were observed with a combination of meprobamate and pentaerythritol tetranitrate [EQUANITRATE] in the patients studied."

For further information on the limitations, administration, and prescribing of EQUANITRATE, see descriptive literature or current direction circular.

†Russek, H.I.: Am J. Cardiol. *3*:547 (April) 1959.

Supplied: EQUANITRATE 10 (200 mg. meprobamate, 10 mg. pentaerythritol tetranitrate), white oval tablets, vials of 50. EQUANITRATE 20 (200 mg. meprobamate, 20 mg. pentaerythritol tetranitrate), yellow oval tablets, vials of 50.

Wyeth Laboratories Philadelphia 1, Pa.

Wyeth SERVICE TO MEDICINE

Equanitrate®

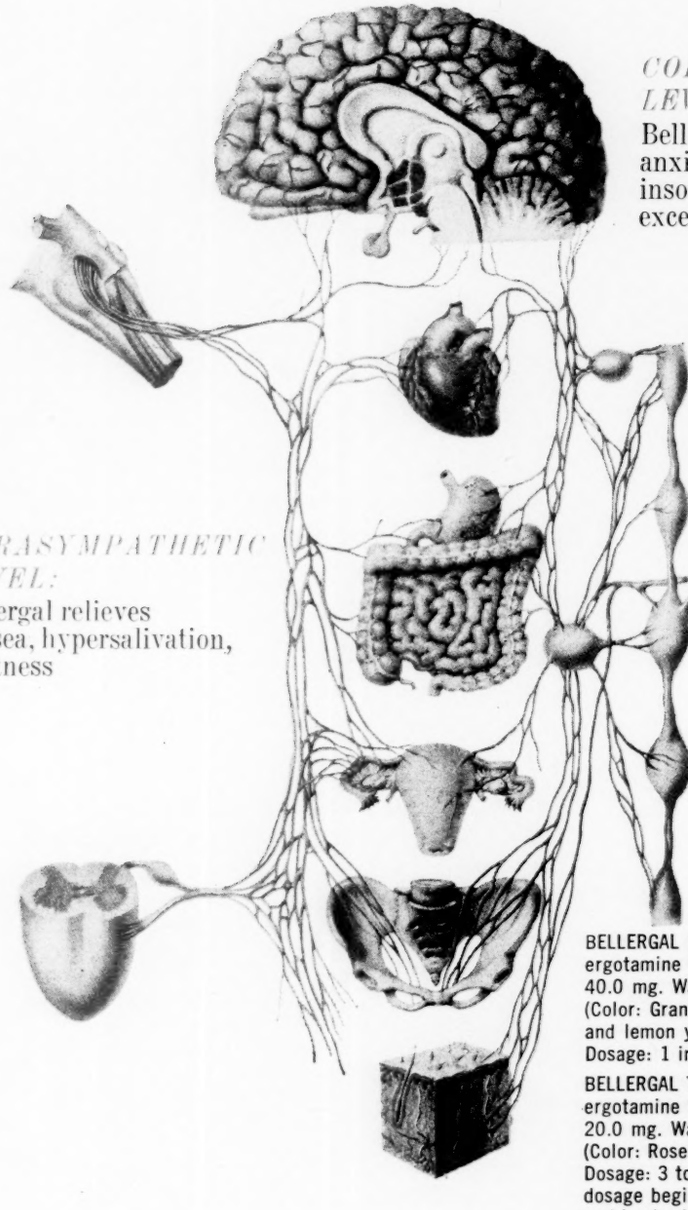
Meprobamate and Pentaerythritol Tetranitrate, Wyeth

Menopausal distress: a syndrome involving all three levels of the autonomic nervous system

for functional disorders of the menopause **Bellergal®** *SPACETABS®*

stabilizes the entire autonomic nervous system

(without disturbing endocrine balance)



CORTICAL LEVEL:
Bellergal relieves anxiety, irritability, insomnia, headache, excessive fatigability

SYMPATHETIC LEVEL:
Bellergal relieves hot flashes, palpitations, tachycardia, tremor, sweats

PARASYMPATHETIC LEVEL:
Bellergal relieves nausea, hypersalivation, faintness

SANDOZ

BELLERGAL SPACETABS—Bellafoline 0.2 mg., ergotamine tartrate 0.6 mg., phenobarbital 40.0 mg. Warning: May be habit forming. (Color: Granular pattern of green, apricot and lemon yellow; compressed.)
Dosage: 1 in the morning, and 1 in the evening.

BELLERGAL TABLETS—Bellafoline 0.1 mg., ergotamine tartrate 0.3 mg., phenobarbital 20.0 mg. Warning: May be habit forming. (Color: Rose beige, sugar-coated.)
Dosage: 3 to 4 daily. In more resistant cases, dosage begins with 6 tablets daily and is slowly reduced.



They Bounce Back Fast with TYLENOL®

acetaminophen

antipyretic / analgesic

He's the same young patient who was feverish and listless yesterday—Tylenol quickly brings fever and discomfort under control.

Tylenol is safe, exceptionally free from side effects[1,2]...well tolerated by children.[1]

AFTER T & A, the gentle antifebrile-analgesic action of Tylenol reduces restlessness, aids recuperation.

TYLENOL ELIXIR—120 mg. (2 gr.) per 5 cc.; 4 and 12 fl. oz. bottles
TYLENOL DROPS—60 mg. (1 gr.) per 0.6 cc.; 15 cc. bottles with calibrated droppers

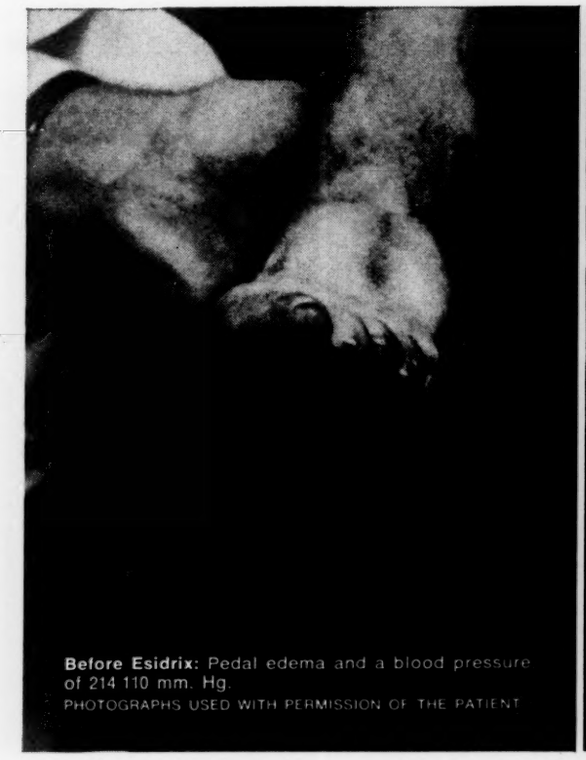
Also available for adults and older children:
Tablets TYLENOL—5 gr. (300 mg.)

McNEIL

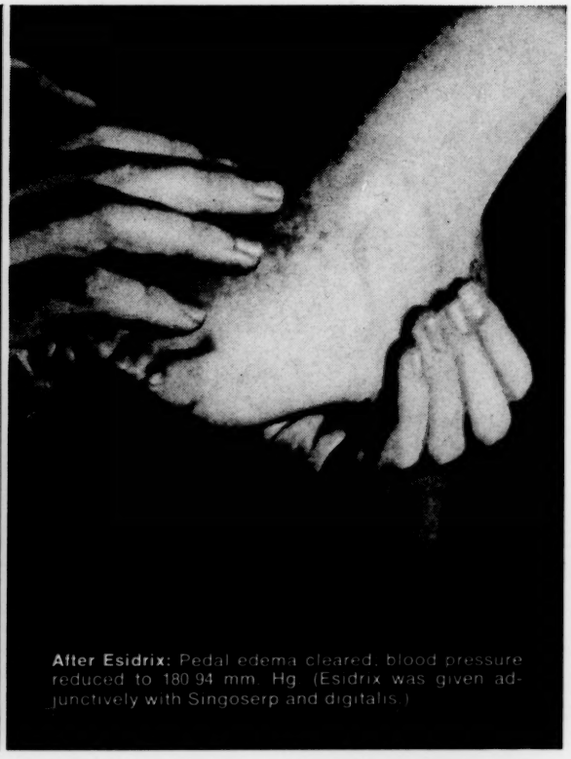
McNeil Laboratories, Inc., Fort Washington, Pa.

1. Cornely, D. A., and Ritter, J. A.: N-acetyl-p-aminophenol (Tylenol Elixir) as a Pediatric Antipyretic-Analgesic, J.A.M.A. *160*:1219-1221 (April 7) 1956.
2. Mintz, A. A.: Management of the Febrile Child, J. Ky. Acad. Gen. Pract. *5*:26-31 (January) 1959.

measurable benefits in edema and hypertension



Before Esidrix: Pedal edema and a blood pressure of 214 110 mm. Hg.
PHOTOGRAPHS USED WITH PERMISSION OF THE PATIENT



After Esidrix: Pedal edema cleared, blood pressure reduced to 180 94 mm. Hg. (Esidrix was given adjunctively with Singoserp and digitalis.)

plus more built-in potassium protection than any other diuretic-antihypertensive

Esidrix-K®

50/1000 Tablets



Supplied: ESIDRIX-K 50/1000 Tablets (white, coated), each containing 50 mg. Esidrix and 1000 mg. potassium chloride (equivalent to 524 mg. potassium).

Also available: ESIDRIX-K 25/500 Tablets (off-white, coated), each containing 25 mg. Esidrix and 500 mg. potassium chloride. ESIDRIX Tablets, 50 mg. (yellow, scored) and 25 mg. (pink, scored).

For complete information about Esidrix and Esidrix-K (including dosage, cautions, and side effects), see current Physicians' Desk Reference or write CIBA, Summit, N. J.

ESIDRIX● (hydrochlorothiazide CIBA)
SINGOSERP● (syrosingopine CIBA)

C I B A Summit, N. J.

2/2989MK

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ITS
SIMPLEST
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ESTOMUL™

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ONE

ESTOMUL—Liquid and Tablets

RESULTS

- RELIEVES SPASM AND REDUCES MOTILITY
- RETARDS ACID PRODUCTION
- PROMPT REDUCTION OF PAIN
- RAPID AND PROLONGED NEUTRALIZATION OF GASTRIC HYDROCHLORIC ACID TO DESIRABLE pH LEVEL
- COATS AND PROTECTS GASTRIC MUCOSA
- INHIBITS EROSION OF MUCOSA

ACTI

- ANTICHOLINERGIC
  orphenadrine hydrochloride
  ANTISPASMODIC

  ANTISECRETORY
- TOPICAL ANESTHETIC
  orphenadrine hydrochloride
- ANTACID
  aluminum hydroxide-magnesium carbonate co-precipitate
- DEMULCENT
  bismuth aluminate
- ANTIPEPTIC
  bismuth aluminate

COMPLETE THERAPEUTIC PROTECTION



ALL DESIRABLE ACTIONS IN ONE MEDICATION

Only ONE prescription to write

FORMULATION

Each ESTOMUL TABLET contains:

Orphenadrine HCl
(2-dimethylaminoethyl (2-methylbenzhydryl) ether
Bismuth aluminate
Magnesium oxide
Aluminum hydroxide } Co-Precipitate
Magnesium carbonate }

INDICATIONS:

Peptic Ulcer:
Duodenal Marginal
Gastric Esophageal
Hyperacidity and dyspepsia
Heartburn

DOSAGE:

LIQUID and TABLETS: 1 or 2 tablespoons or 1 or 2

SIDE ACTIONS:

Doses in excess of 6 tablets or 6 tablespoons daily may
blurring of vision.

CONTRAINDICATIONS:

ESTOMUL should not be used in patients with organic
in patients with renal impairment or insufficiency. Relative
prostatic hypertrophy which may lead to urinary bladder

AVAILABILITY:

Tablets – Bottles of 100.
Liquid – Bottle of 12 fluid oz.

Riker

MID-MORNING HUNGER-FATIGUE *calls for a* HEARTY BREAKFAST





TEEN-AGERS *need high quality proteins*

MEAT *is a high quality protein*

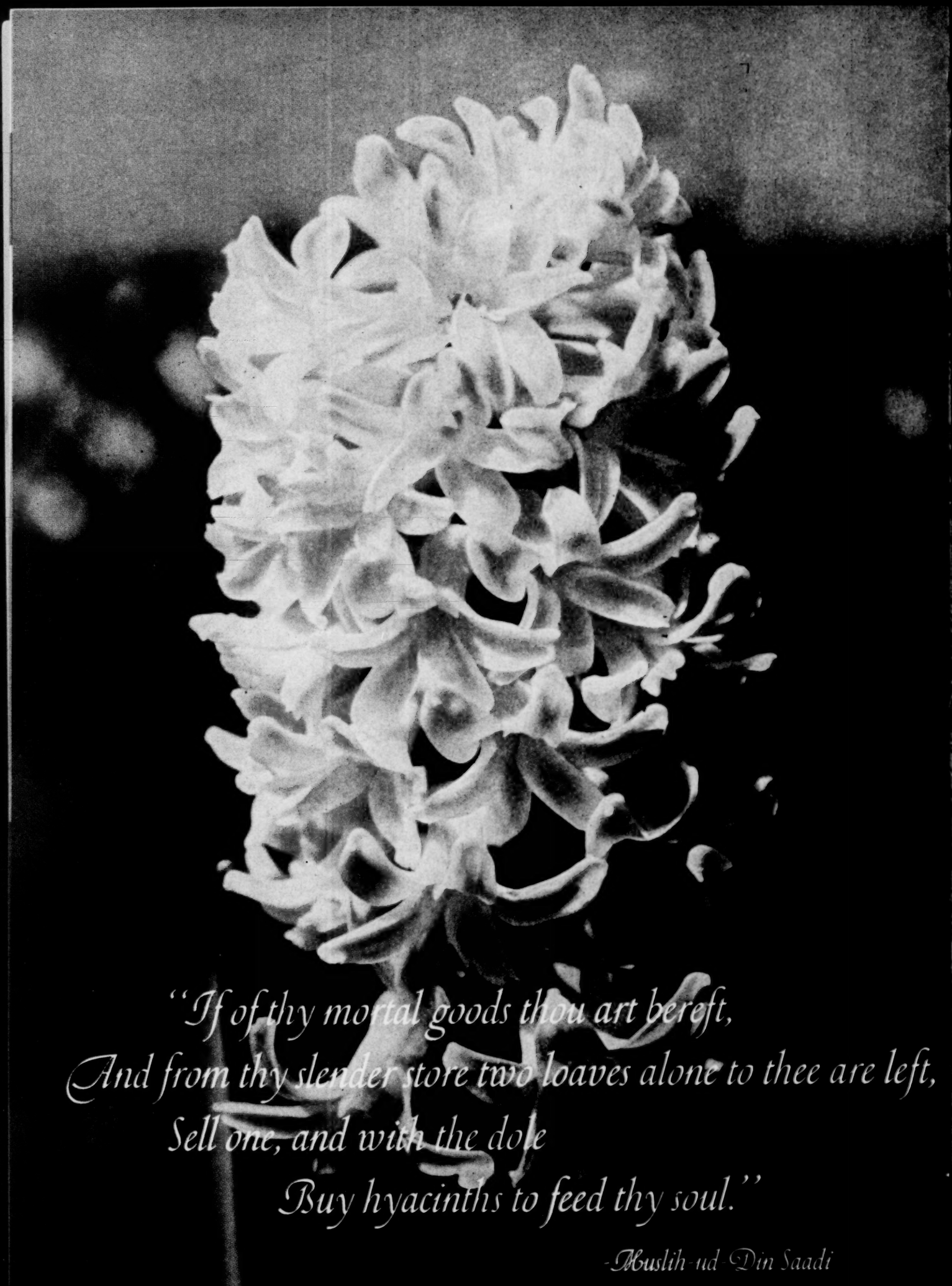
. . . studies have supported the view that the calorie intake and ratio of carbohydrate to protein in the breakfast meal influence the post-prandial blood glucose curve, with the higher protein intakes being associated with lower peaks and slower return to fasting levels. (1, 2, 3)

While the source of calories is not critical—an intake of 15 or more gm. of protein slows the blood sugar fall and may be associated with a sense of well-being lacking in low protein breakfasts. (4)

1. Tuttle, Wilson, and Daum: J. Appl. Physiol., **1**, 545, 1949
2. Orent-Keiles, and Hallman: Circ. No. 827, 1949, U.S. Dept. Agric., Washington, D.C.
3. Coleman, Tuttle, and Daum: J. Am. Dietetic Assoc., **29**, 239, 1953.
4. Maurice E. Shils—Mod. Nut. in Health & Disease, Ch. 37, 1960

AMERICAN MEAT *INSTITUTE*

MAIN OFFICE, CHICAGO • MEMBERS THROUGHOUT THE NATION


"If of thy mortal goods thou art bereft,
And from thy slender store two loaves alone to thee are left,
Sell one, and with the dole
Buy hyacinths to feed thy soul."
-Muslih-ud-Din Saadi

Upjohn

75th year

Man does not live by bread alone.
If he did, medicine would be purely a science, concerned only with "bread to nourish the body."

Thoughtful physicians have long recognized the equal essentiality of "hyacinths to feed the soul." This is the *art* of medicine.

If yours is a typical practice, many of the patients who come to you have no demonstrable somatic pathology. Yet their symptoms often are myriad: low back pain, recurrent headaches, insomnia, anorexia, chronic fatigue, apathy, inability to concentrate, "blues."

While tranquilizers may be indicated in some of these patients, many of them are candidates for the simple psychomotor stimulating effect of Monase. Tests in more than 4,000 patients justify the expectation that Monase will enable many of these patients to sleep better, eat better, and feel better.

For the 4 out of 10 patients with no demonstrable pathology,† consider

Monase*



*TRADEMARK, REG. U.S. PAT. OFF.
†ESTIMATED AVERAGE IN GENERAL PRACTICE
COPYRIGHT, 1961, THE UPJOHN COMPANY

Description: Monase is etryptamine acetate, a unique non-hydrazine compound, developed in the Upjohn Research Laboratories.

Indications: Various depression states: manic-depressive reaction, depressed type; involutional psychotic reactions with depressed features; psychotic depressed reactions; psychoneurotic depressive reactions; psychiatric disorders with prominent depressive symptoms or features; transient situational personality disorders with pathological depressive features.

Dosage: 30 mg. daily in divided doses. Initial benefit may be observed within 2-3 days, but maximum results may not be apparent until after 2 or more weeks. Adjustment of dose to individual response should be effected in increments or decrements of 15 mg. daily at weekly intervals. The daily maintenance dose ranges between 15 and 45 mg. In schizophrenics, 30 mg. daily may be useful as an adjunct in activating these patients or brightening their mood.

Contraindications and Precautions: There are no known absolute contraindications to Monase therapy. However, the drug should be used with caution in schizoid or schizophrenic patients, paranoids, and in patients with intense anxiety, as it may contribute to the activation of a latent or incipient psychotic process. Patients with suicidal tendencies should be kept under careful observation during Monase therapy until such time as the self-destructive tendencies are brought under control.
Patients who are on concomitant antihypertensive therapy should be watched carefully for possible potentiation of hypotensive effects. Added caution should be employed in patients with cardiovascular disease in view of the occasional occurrence of postural hypotension, and the possibility of increased activity as a result of a feeling of increased well being.
Despite the fact that liver damage or blood dyscrasias have not been reported in patients receiving Monase, as is the case with any new drug, patients should be carefully observed for the development of these complications. Monase should probably not be used in patients with a history of liver disease or abnormal liver function tests. Also the usual precautions should be employed in patients with impaired renal function, since it is possible that cumulative effects may occur in such patients.
Monase should be employed with caution in patients with epilepsy since the possibility exists that the epileptic state may be aggravated. Also because of its autonomic effects, therapy with Monase may aggravate glaucoma or may produce urinary retention. Monase must not be administered concomitantly with imipramine. In patients receiving Monase caution should be employed in administering the following agents or related compounds in view of possible lowering of the margin of safety: meperidine, local anesthetics (procaine, cocaine, etc.), phenylephrine, amphetamine, alcohol, ether, barbiturates or histamine.

Toxicity and Side Effects: The side effects observed in patients on Monase therapy in general have been mild and easily managed by symptomatic therapy or dose reduction. If such side effects persist of are severe, the drug should be discontinued. Alterations in blood pressure, usually in the form of postural hypotension, or more rarely, an elevation of blood pressure have been reported. Other side effects include allergic skin reactions and drug fever and those that appear to be dose related since they are more likely to occur when the daily dose exceeds 60 mg. These are nausea and gastrointestinal upset, headache, vertigo, palpitation, dryness of the mouth, blurred vision, over-stimulation of the central nervous system, restlessness, insomnia, paradoxical somnolence and fatigue, muscle weakness, edema, and sweating. Following sudden withdrawal of medication in patients receiving high doses for a prolonged period. There may occur a "rebound" withdrawal effect which is characterized by headache, central nervous system hyperstimulation and occasionally hallucinations.

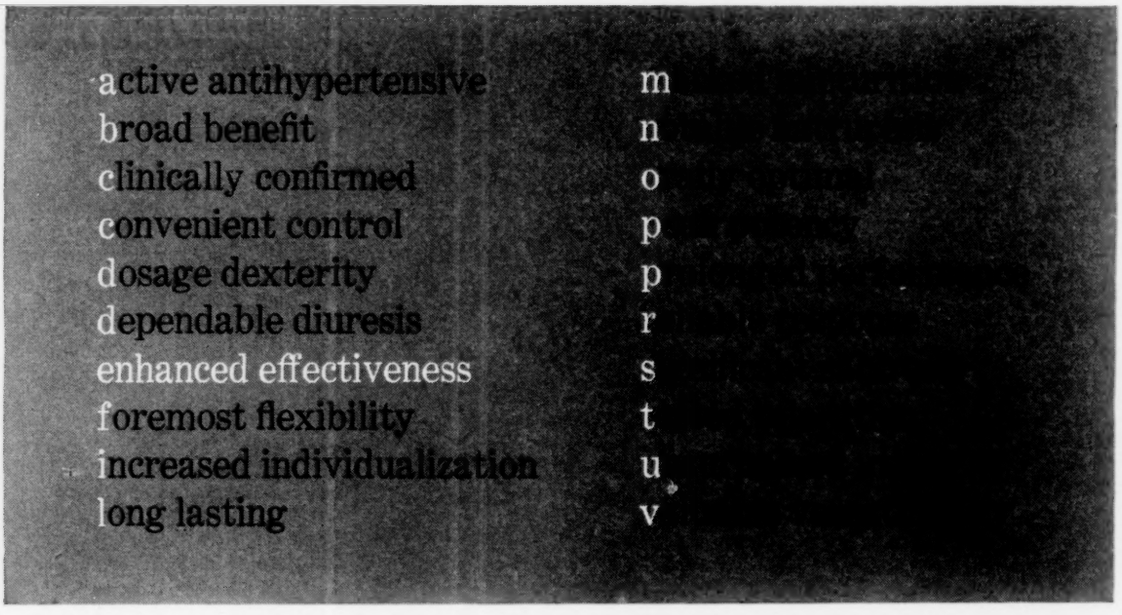
Supplied: 15 mg. compressed tablets in bottles of 100 and 500.

new Renese®

POLYTHIAZIDE

1 mg. 2 mg. 4 mg.

A MORE CLINICALLY USEFUL
DIURETIC/ANTIHYPERTENSIVE



"enhanced effectiveness"

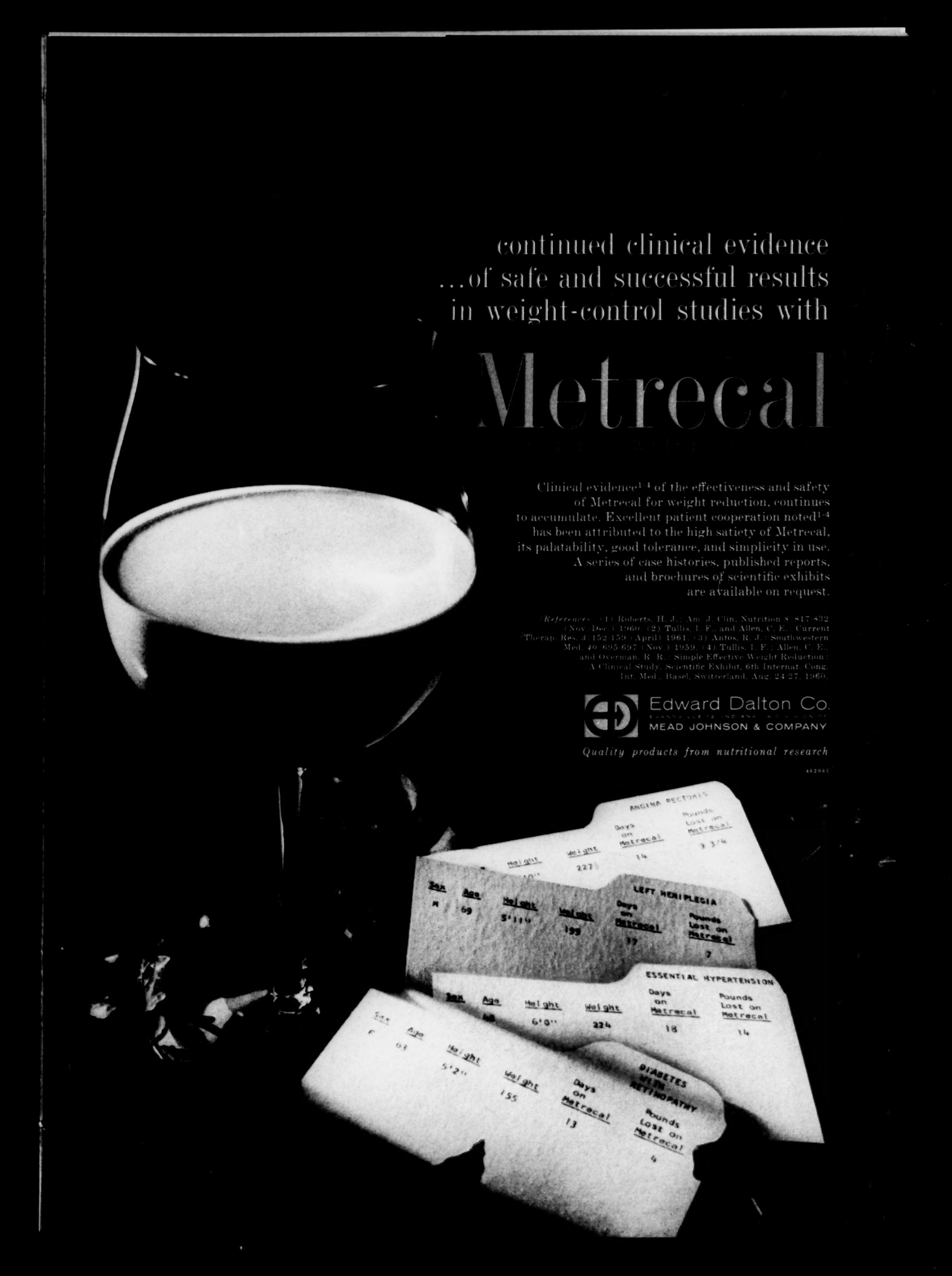
– In a 24-hour dose response study, RENESE produced a greater increase in sodium excretion than has been shown with four other currently available thiazides. In a controlled study[1] of patients with hypertensive cardiovascular disease, free of detectable edema, the 24-hour urinary volume increased by an average of 1.8 liters with an accompanying average weight reduction of 1.4 Kg. following a single 8 mg. dose of RENESE. The enhanced effectiveness of RENESE may produce response where previous therapy has failed or improve the response to present therapy.

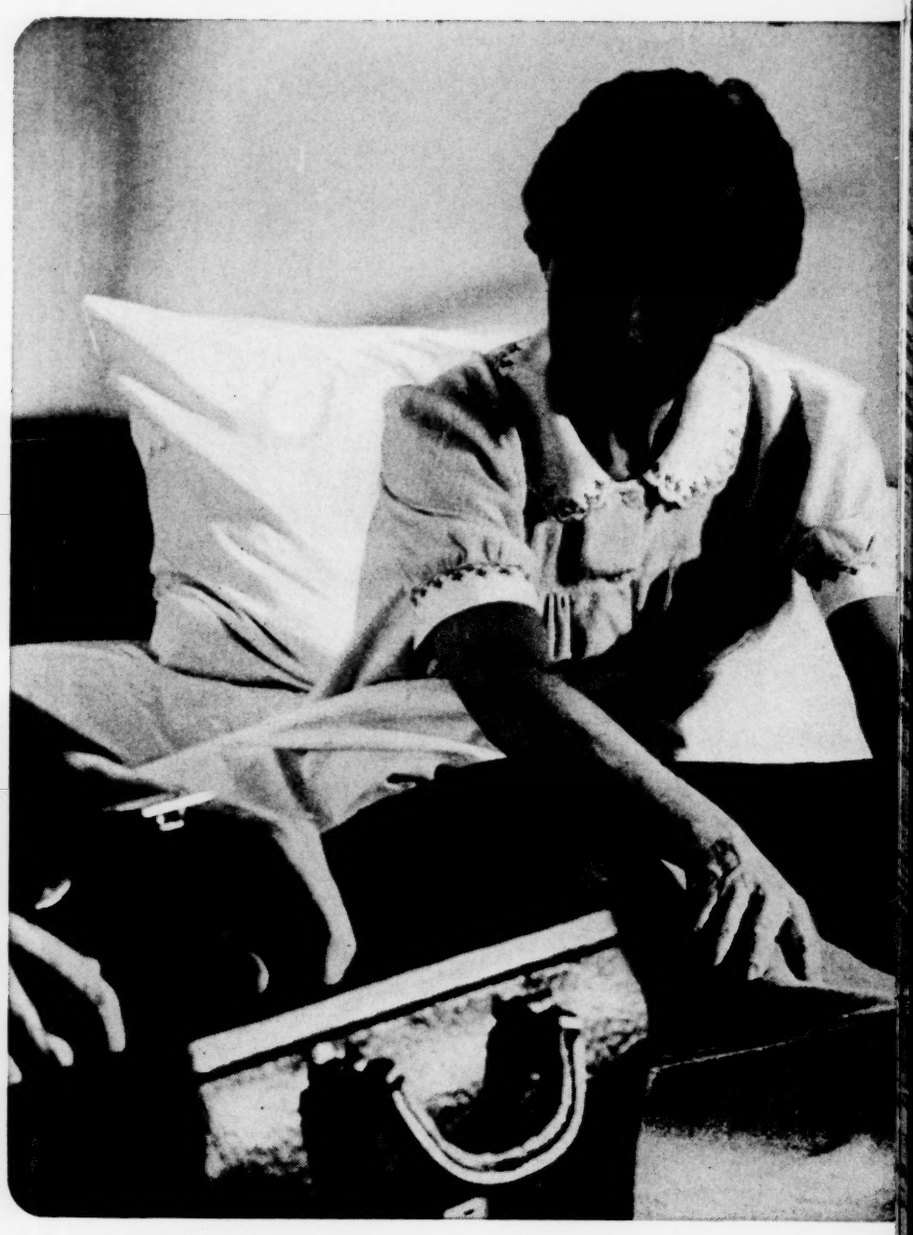
1. Ford, R. V.: Current Therap. Res. 3:320, July, 1961

Pfizer *Science for the world's well-being®*

PFIZER LABORATORIES Division,
Chas. Pfizer & Co., Inc. New York 17, New York

FOR PRODUCT INFORMATION TURN TO PAGE 96


continued clinical evidence
...of safe and successful results
in weight-control studies with
Metrecal
Clinical evidence[1-4] of the effectiveness and safety of Metrecal for weight reduction, continues to accumulate. Excellent patient cooperation noted[1-4] has been attributed to the high satiety of Metrecal, its palatability, good tolerance, and simplicity in use. A series of case histories, published reports, and brochures of scientific exhibits are available on request.
References: (1) Roberts, H. J.: Am. J. Clin. Nutrition 8:817-832 (Nov.-Dec.) 1960. (2) Tullis, J. F., and Allen, C. E.: Current Therap. Res. 3:152-159 (April) 1961. (3) Antos, R. J.: Southwestern Med. 40:695-697 (Nov.) 1959. (4) Tullis, J. F.; Allen, C. E.; and Overman, R. R.: Simple Effective Weight Reduction: A Clinical Study, Scientific Exhibit, 6th Internat. Cong. Int. Med., Basel, Switzerland, Aug. 24-27, 1960.
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Quality products from nutritional research
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ANGINA PECTORIS
Days on Metrecal
Pounds Lost on Metrecal
14
3 3/4
LEFT HEMIPLEGIA
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Age
69
Height
5'11"
Weight
199
Days on Metrecal
17
Pounds Lost on Metrecal
7
ESSENTIAL HYPERTENSION
Sex
Age
Height
6'0"
Weight
224
Days on Metrecal
18
Pounds Lost on Metrecal
14
DIABETES WITH RETINOPATHY
Sex
Age
Height
5'2"
Weight
155
Days on Metrecal
13
Pounds Lost on Metrecal
4



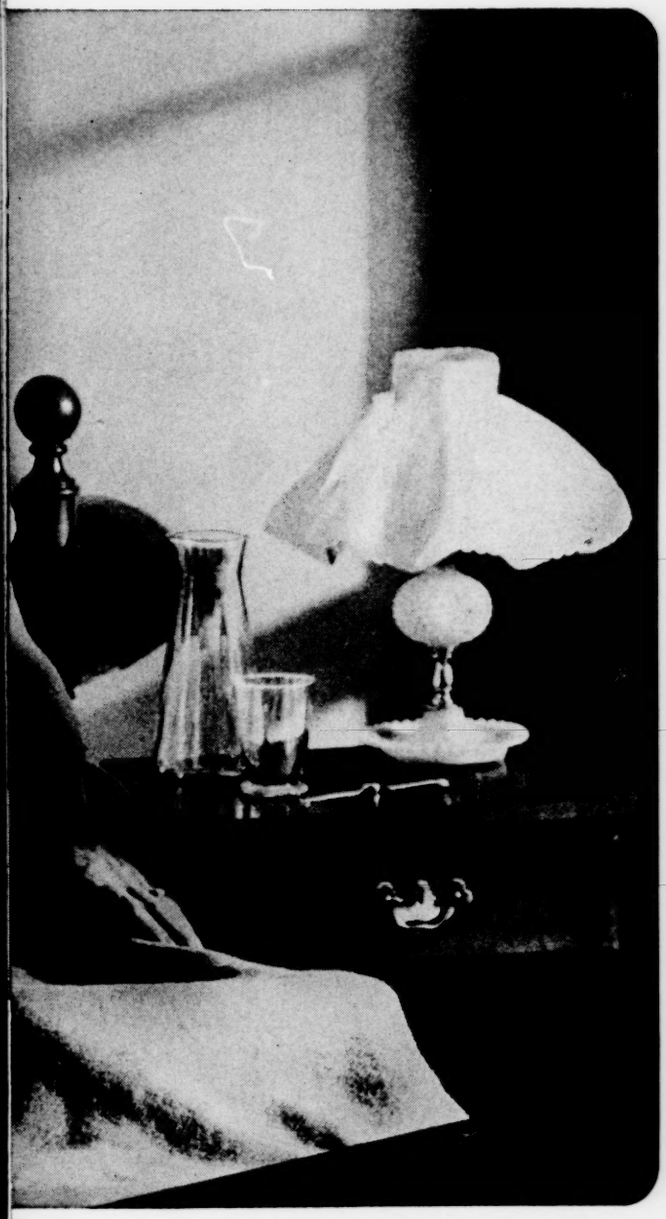
CALL ON THIS ORAL PENICILLIN FOR PROMPT, POTENT ANTIBIOTIC ACTION

PEN·VEE® K

Liquid: Penicillin V Potassium for Oral Solution, Wyeth
Tablets: Penicillin V Potassium, Wyeth

produces high penicillin blood levels

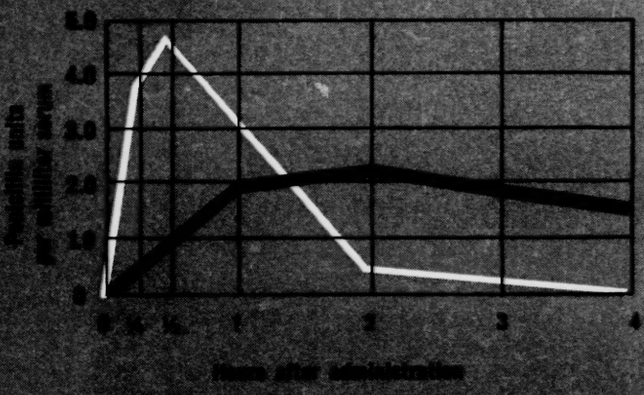
- easy-to-take Tablets or Liquid
- readily absorbed from the GI tract
- avoids pain, bother, and risk of injections
- palatable and well tolerated
- for all infections responsive to oral penicillin
- and for some usually requiring parenteral penicillin



A potent oral penicillin for high therapeutic efficacy

You can prescribe PEN•VEE K for any and all infections caused by penicillin-susceptible organisms. It is a reliable and predictable antibiotic. Demonstrable blood levels occur within 15 minutes after ingestion; peak blood levels within 30 minutes. PEN•VEE K is markedly effective for treatment and prophylaxis of common bacterial infections, including hemolytic streptococcal infections, certain staphylococcal infections, and pneumococcal and gonococcal infections.

Serum concentrations— oral and parenteral penicillin



Hours after administration

⊖ Potassium penicillin V, 250 mg. (400,000 units) oral tablet. Average of 40 adult subjects.*

Procaine penicillin G (600,000 units) intramuscular injection. Average of 15 subjects.†

Palatable, convenient, well tolerated

PEN•VEE K is palatable, convenient (tablet or liquid), and well tolerated. These factors encourage good patient cooperation, which helps promote rapid recovery.

References: 1. Peck, F.B., Jr., and Griffith, R.S.: Antibiotics Annual 1957-58, Medical Encyclopedia, Inc., p. 1004. 2. White, A.C., et al.: Antibiotics Annual 1956-57, Medical Encyclopedia, Inc., p. 490.

For further information on limitations, administration, and prescribing of PEN•VEE K, see descriptive literature or current Direction Circular.

Wyeth Laboratories Philadelphia 1, Pa.

Wyeth®



The Clinical Study:[1]

38 patients, male and female, all of whom gained weight on a previous diet regimen.

The Rx:

Desoxyn Gradumet, 10 or 15 mg., *once daily,* in the morning.

The Clinical Result:

The entire group averaged a weight loss of 15 pounds between two and 12 months of treatment. Eight women, weighing between 178 and 269 pounds, took Desoxyn for six months. They averaged a loss of 34 pounds. The heaviest woman lost 57 pounds, while another lost 71 pounds.

All the results weren't good, of course. One male actually gained seven pounds after four months. And there were two other patients whose weight remained unchanged after four and six months of treatment.

A Sidelight:

The clinician noted that there was an occasional finding of tablets in stools. He wrote: "A certain doubt existed in our minds that this residual tablet did not contain amphetamine. We arranged



for analysis for four of such tablets in patients taking 15 mg. and found that in each instance, there was no trace of amphetamine."

The Conclusion:

"Desoxyn has the best patient acceptance of any of the medications we have used as an appetite suppressant . . . It is our impression that Desoxyn Gradumet does slowly release amphetamine and thus, reduce side effects that are a problem. It is likewise our impression that the medication is a definite aid in weight reduction and has a high patient acceptability. We are at present using this drug in our practice as a routine adjutant to a weight reduction program."

1. Cole, R. E., Communication to Medical Department, Abbott Laboratories, 1961.

The Drug:

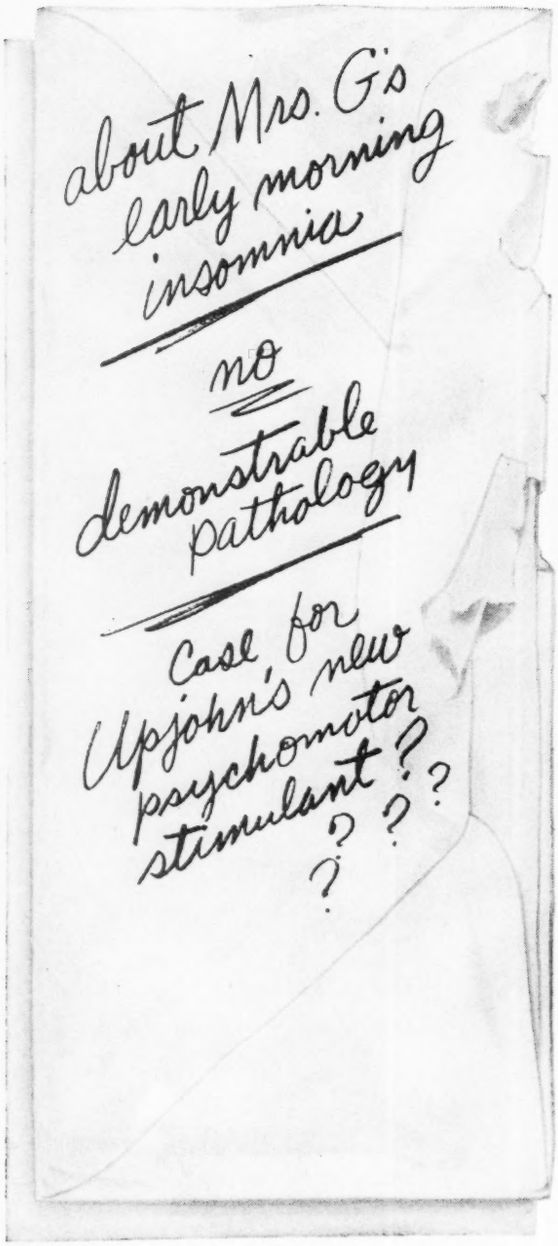
DESOXYN Gradumet®

(Methamphetamine Hydrochloride in Long-Release Dose Form*)

All-Day Appetite Control from a Single Oral Dose—5, 10 or 15 mg.

ABBOTT

*U.S. PAT. NO. 2,987,445 110242



FOR COMPLETE DETAILS ON

Monase*

*Trademark, Reg. U.S. Pat. Off. – brand of etryptamine acetate

SEE PAGE 40–41

Upjohn

75th year

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The American Journal of Medicine

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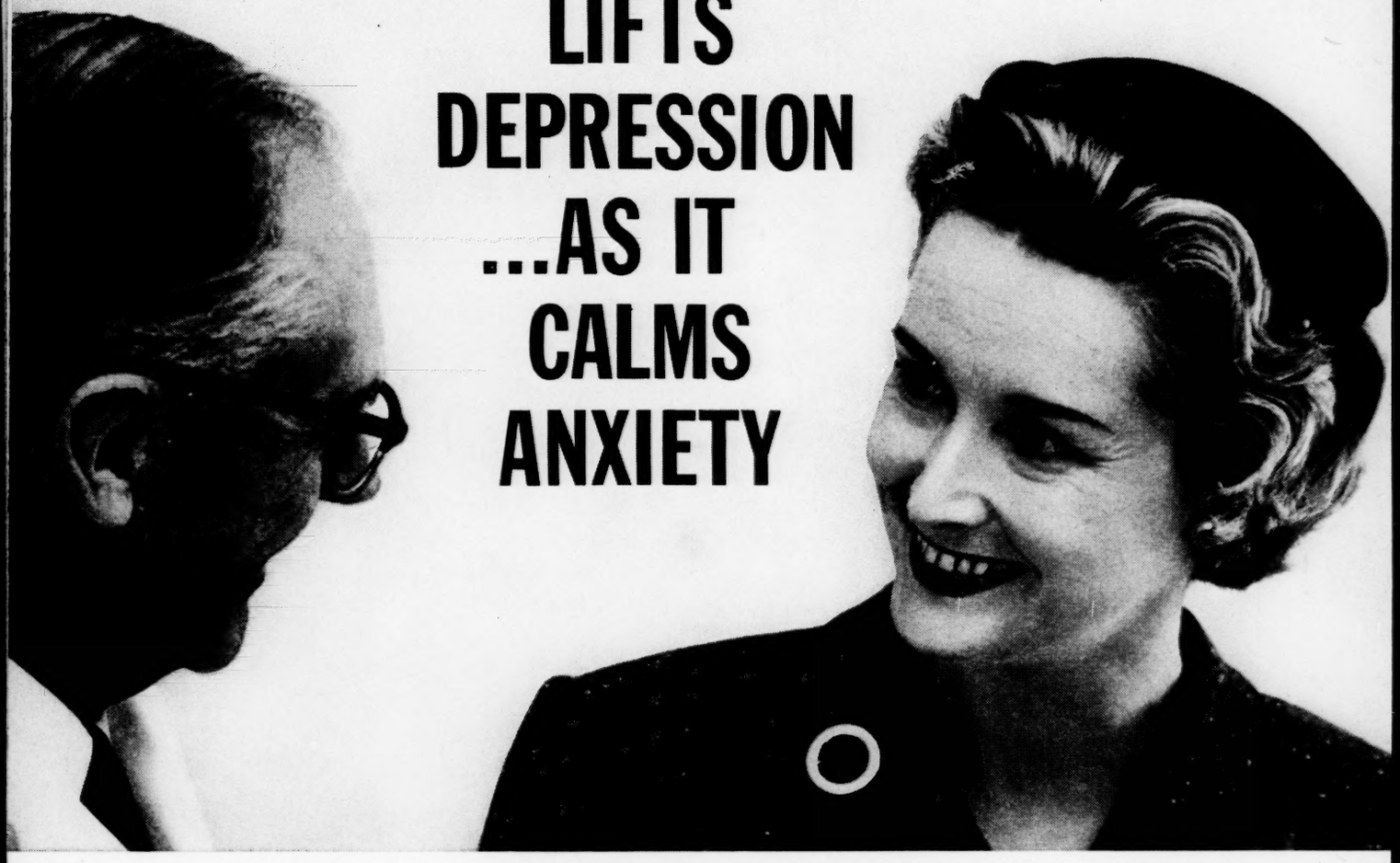
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LIFTS DEPRESSION ...AS IT CALMS ANXIETY



"I feel like my old self again!" Thanks to your balanced Deprol therapy, normal drive and interest have replaced her emotional fatigue.

Brightens up the mood, brings down tension

Deprol's balanced action avoids "seesaw" effects of energizers and amphetamines. While energizers and amphetamines may stimulate the patient – *they often aggravate anxiety and tension.*

And although amphetamine-barbiturate combinations may counteract excessive stimulation – *they often deepen depression and emotional fatigue.*

These "seesaw" effects are avoided with Deprol. It lifts depression as it calms anxiety – a balanced action that brightens up the mood, brings down tension, and relieves insomnia, anorexia and emotional fatigue.

Acts rapidly – you see improvement in a few days. Unlike the delayed action of most other antidepressant drugs, which may take two to six weeks to bring results, Deprol relieves the patient quickly – *often within a few days.* Thus, the expense to the patient of long-term drug therapy can be avoided.

Compatible with therapy for physical diseases. Deprol can be used safely with specific therapies for cardiovascular, G.I. and upper respiratory conditions. It does not cause liver damage, hypotension or tachycardia.

▲Deprol▲®

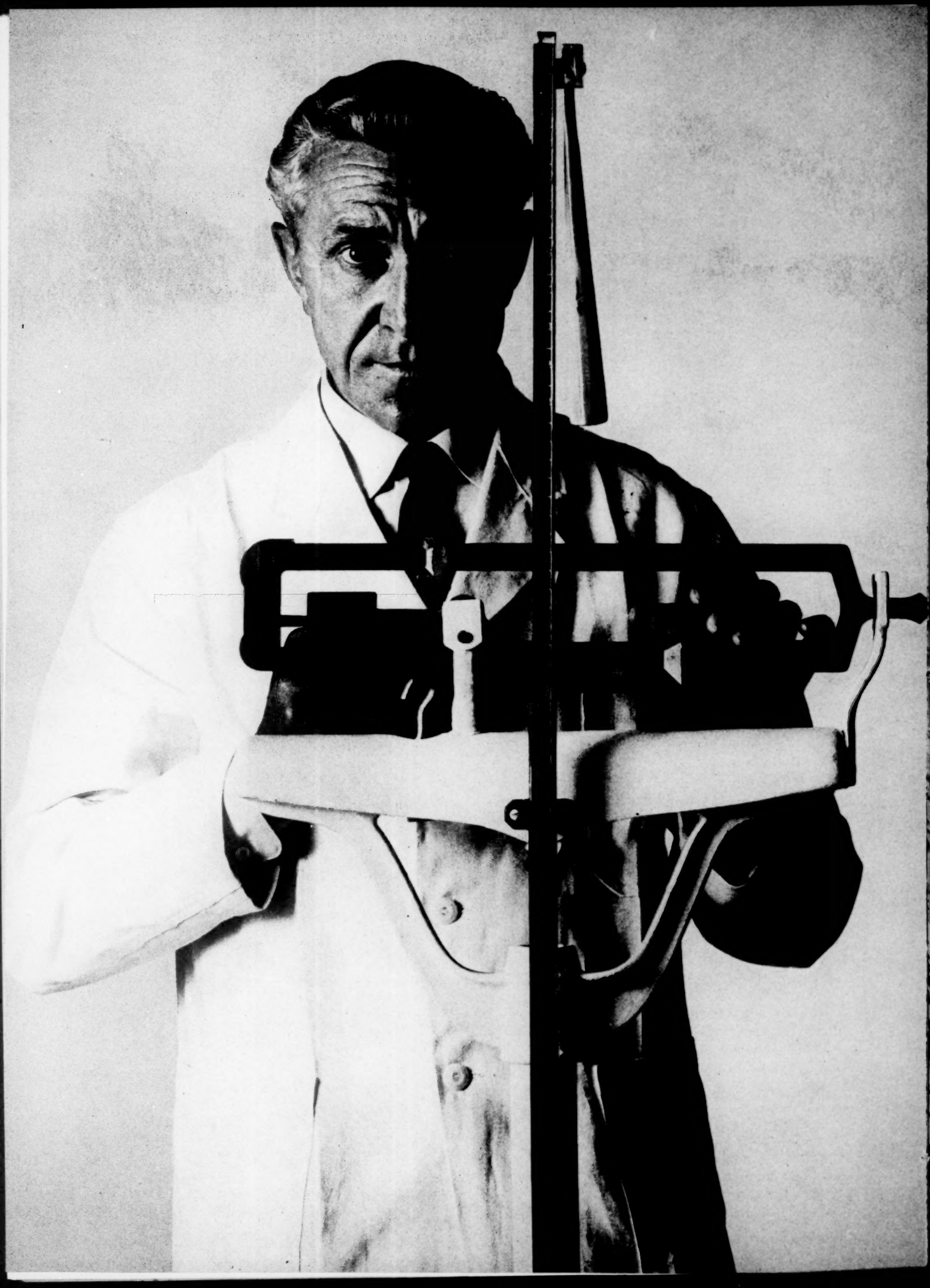
Dosage: Usual starting dose is 1 tablet q.i.d. When necessary, this may be increased gradually up to 3 tablets q.i.d. With establishment of relief, the dose may be reduced gradually to maintenance levels.

Composition: 1 mg. 2-diethylaminoethyl benzilate hydrochloride (benactyzine HCl) and 400 mg. meprobamate.

Supplied: Bottles of 50 light-pink, scored tablets. Write for literature and samples.

WALLACE LABORATORIES / *Cranbury, N. J.*

CD-5852



Can we measure the patient's comfort?

The physician can measure body weight by means of a scale. But he has no instrument—no objective test—for measuring comfort.

For this, he must depend upon his own powers of observation and the patient's own description of how he feels.

Because these are, admittedly, subjective criteria, the validity of results hinges entirely on the experience and objectivity of the investigators involved.

Such well-qualified clinicians have reported that a new corticosteroid developed in the research laboratories of Upjohn actually raises the level of relief obtainable with this type of therapy.

This difference cannot be "proved." It must be seen. And the only practical way for you to do this is to evaluate this new drug critically in your own practice. Please do, at your first opportunity. We are confident that you will be glad you did.

The new corticosteroid from Upjohn research

Alphadrol*

Each tablet contains Alphadrol (fluprednisolone) 0.75 mg. or 1.5 mg.
Supplied in bottles of 25 and 100.

The anti-inflammatory activity of Alphadrol is comparable to the best effects obtained in current practice. Results obtained with Alphadrol have been such as to warrant classifying it among the most efficient steroids now available.

More than twice as potent as prednisolone, Alphadrol exhibits no new or bizarre side effects. Salt retention, edema or hypertension, potassium loss, anorexia, muscle weakness or muscle wasting, excessive appetite, abdominal cramping, or increased abdominal girth have not been a problem.

Indications and effects
The benefits of Alphadrol (anti-inflammatory, antiallergic, antirheumatic, antileukemic, antihemolytic) are indicated in acute rheumatic carditis, rheumatoid arthritis, asthma, hay fever and allergic disorders, dermatoses, blood dyscrasias, and ocular inflammatory disease involving the posterior segment.

Precautions and contraindications
Patients on Alphadrol will usually experience dramatic relief *without* developing such possible steroid side effects as gastrointestinal intolerance, weight gain or weight loss, edema, hypertension, acne or emotional imbalance.

As in all corticotherapy, however, there are certain precautions to be observed. The presence of diabetes, osteoporosis, chronic psychotic reactions, predisposition to thrombophlebitis, hypertension, congestive heart failure, renal insufficiency, or active tuberculosis necessitates careful control in the use of steroids. Like all corticosteroids, Alphadrol is contraindicated in patients with arrested tuberculosis, peptic ulcer, acute psychoses, Cushing's syndrome, herpes simplex keratitis, vaccinia, or varicella.

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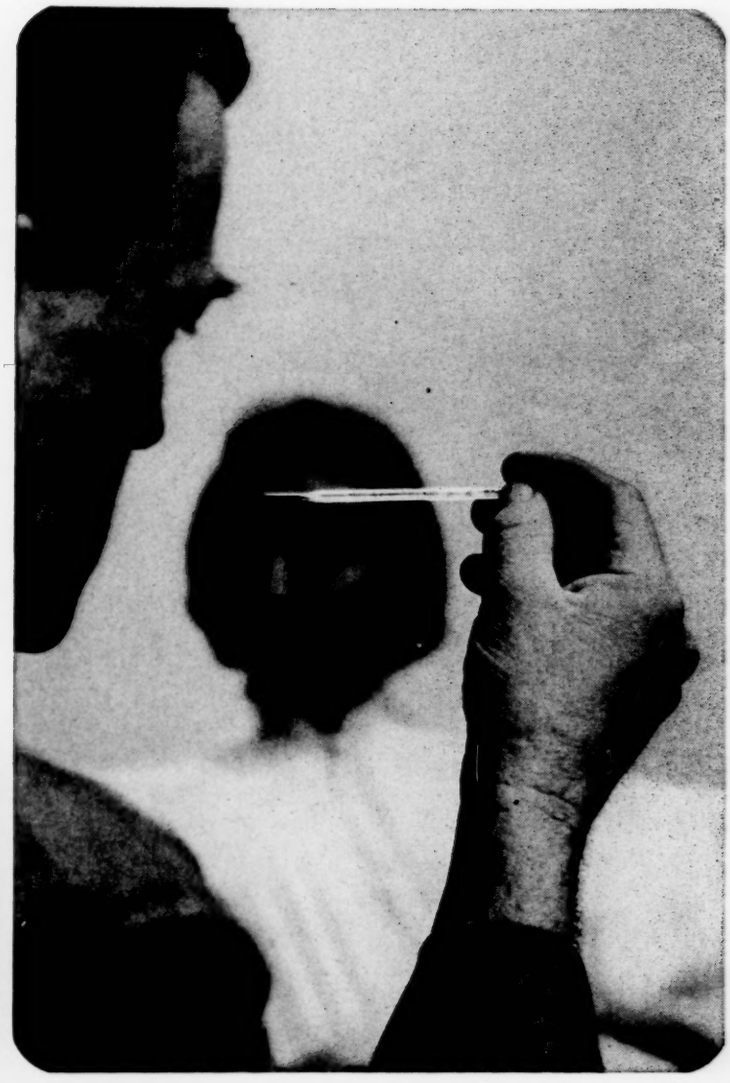
References: (1) Lubowe, I. I.: Antibiot. Med. & Clin. Therap. **4**:81, 1957. (2) Fox, H. H.: Antibiot. Med. **6**:85, 1959. (3) Murphy, J. C.: Rocky Mountain M. J. **55**:53, 1958. (4) Pace, B. F.: Med. Rec. & Ann. **51**:370, 1957.

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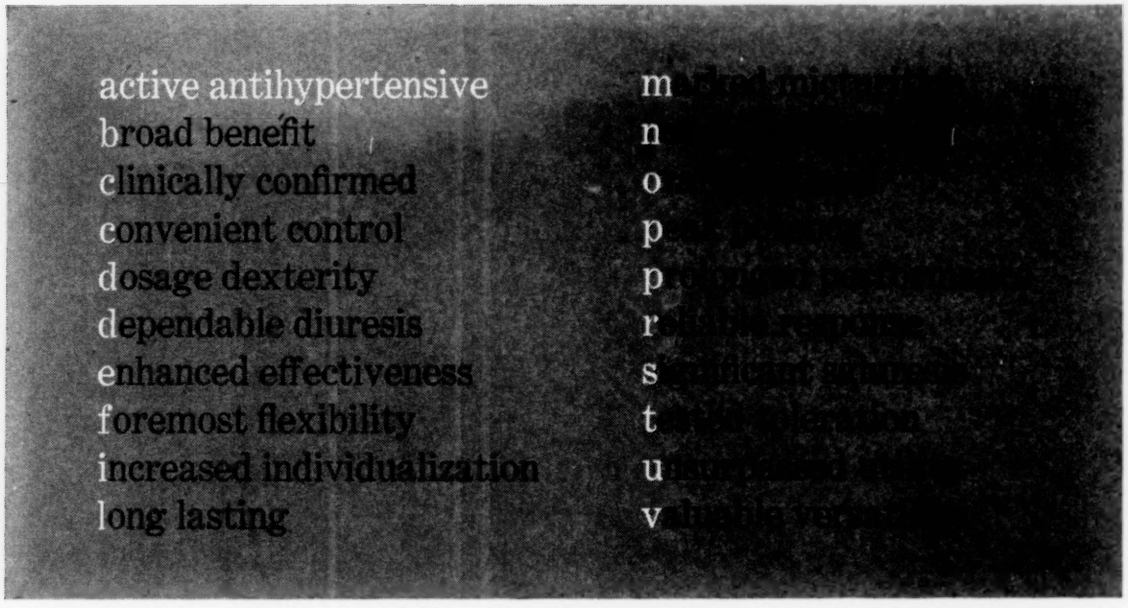
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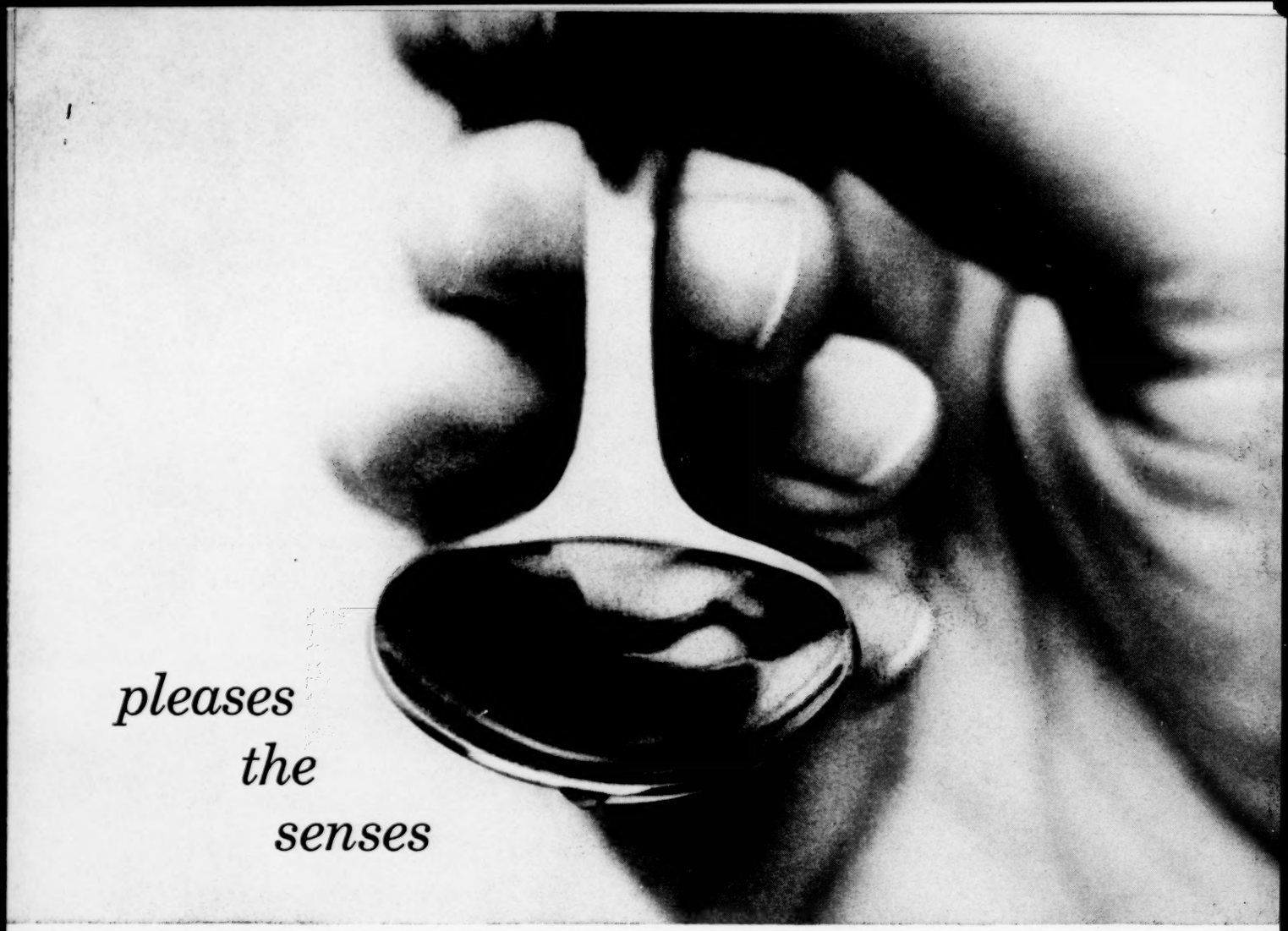
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FOR PRODUCT INFORMATION TURN TO PAGE 96



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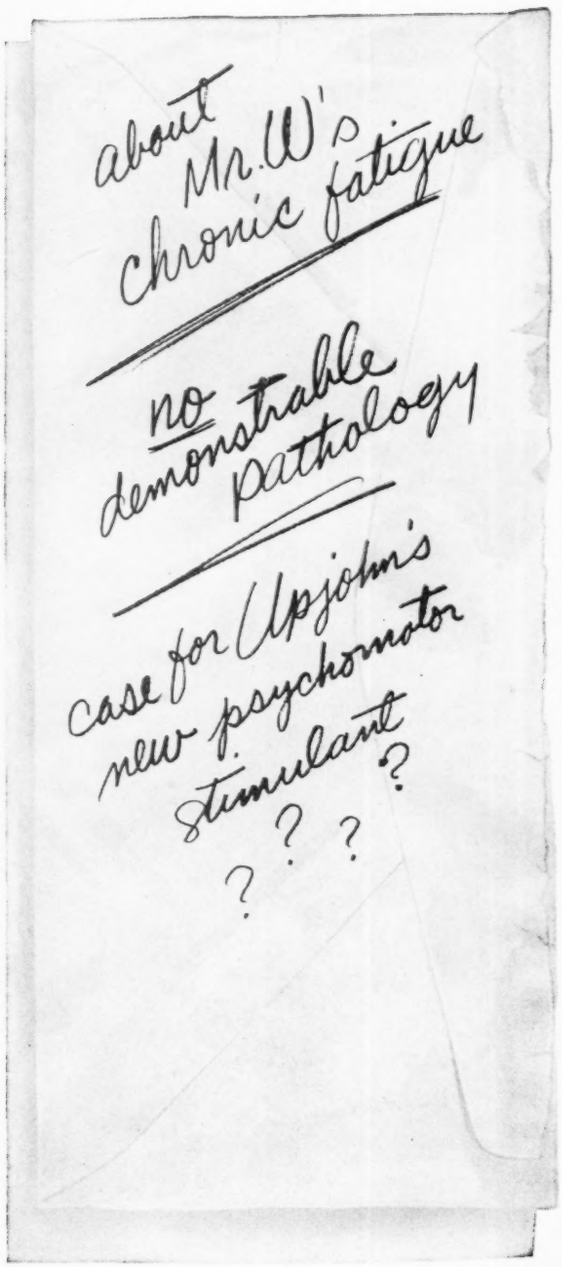
Consider good-tasting Norisodrine Syrup for your next respiratory allergy patient. It can help you manage symptoms that have been troublesome for years.

[1]Frohman, I. P., A New Antitussive Agent, M. Times, 88:924, Aug. 1960.

Norisodrine: Isoproterenol Sulfate, Abbott.

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ONCOGENIC VIRUSES

Ludwik Gross, M.D., F.A.C.P., *Chief Cancer Research Unit, Veterans Administration Hospital, Bronx, N.Y.; Associate Scientist, Sloan-Kettering Institute.*

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In this significant book, the author makes an extensive review of the known oncogenic viruses and discusses the current status of experimental research on virus-caused neoplasms. Historical data on the initial original observations dealing with the establishment of the viral nature of certain animal tumors are included, with particular emphasis placed on developments of the past ten years in this area of research, particularly in the field of mouse leukemia and parotid tumor (polyoma) viruses.

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Editors: S. S. Kety and J. Elkes, *National Institute of Mental Health, Bethesda*

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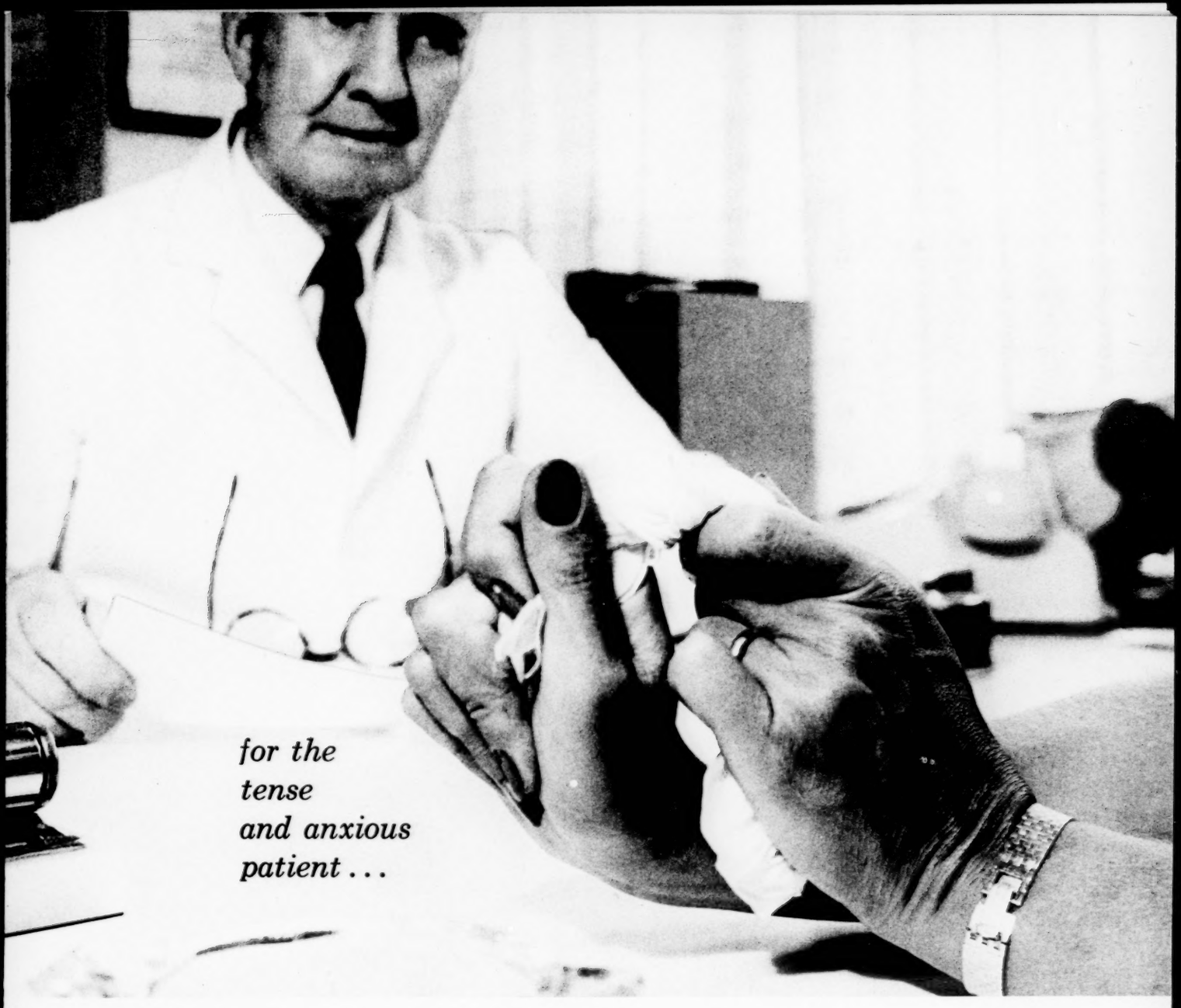
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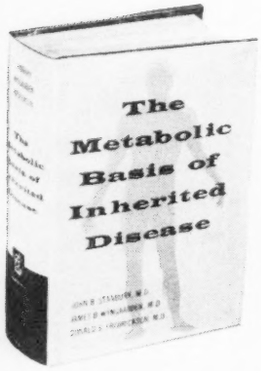
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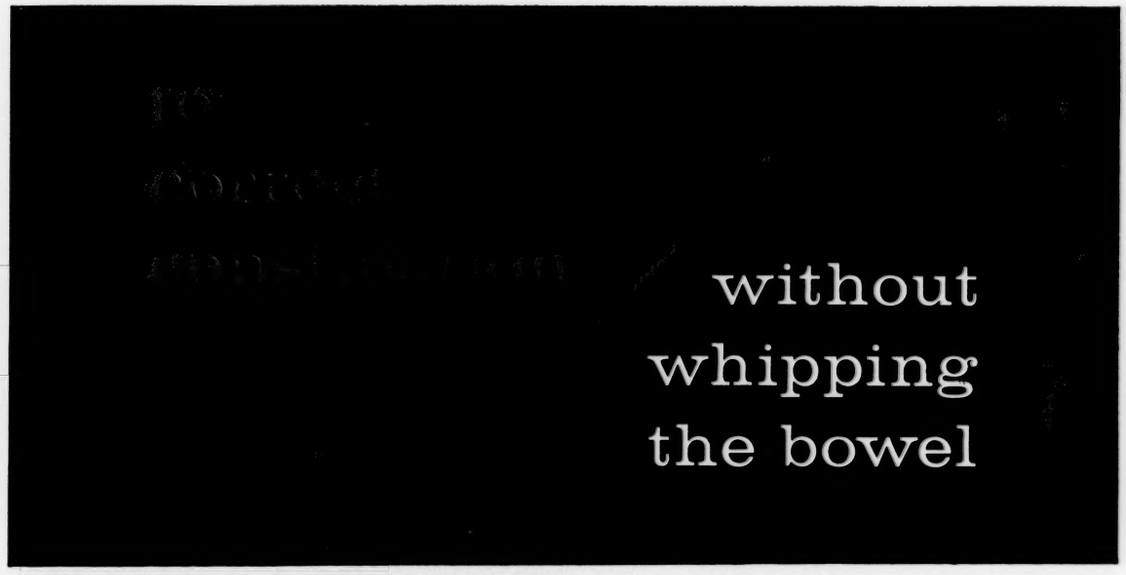
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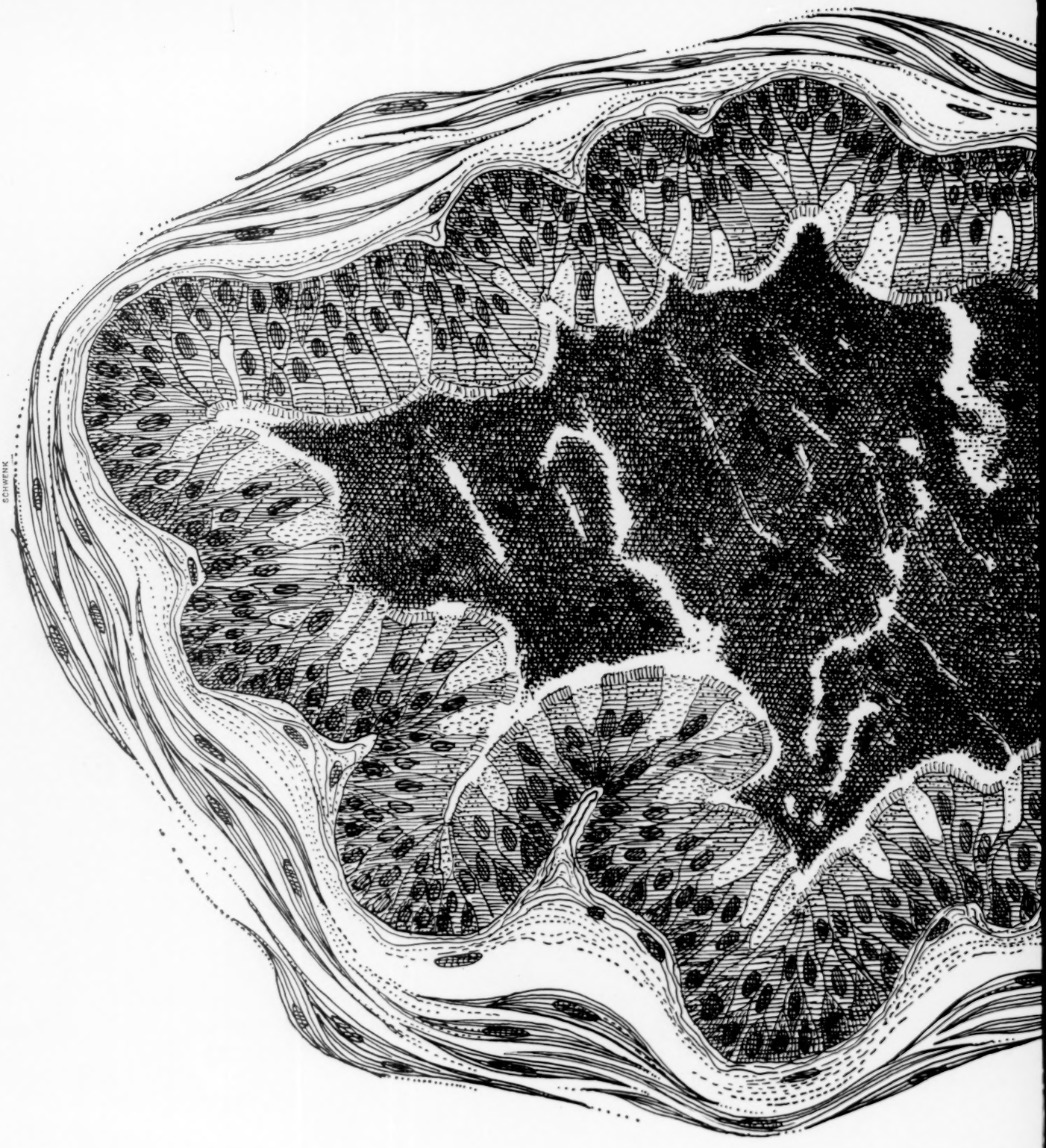
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when **anxiety and tension**

aggravate pain

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Relieves pain, relaxes mind and muscle

- analgesic action to relieve pain
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For your patients suffering pain accompanied by anxiety and tension, EQUAGESIC provides gratifying relief. Potent, non-narcotic analgesia is provided by a combination of the potent analgesic, ethoheptazine citrate, with time-proved aspirin. The muscle-relaxant and anti-anxiety effects of meprobamate, coupled with the analgesic agents provide *analgesia in depth.*

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1. Splitter, S.R.: Current Therapeutic Research *2*:169 (June) 1960. 2. Harsha, W.N.: J. Okla. State Med. Assoc. *54*:12 (Jan.) 1961.

For further information on limitations, administration and prescribing of EQUAGESIC, see descriptive literature or current Direction Circular.

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CYTOXAN®

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CYTOTOXIC AGENT for palliative chemotherapy of certain types of malignant neoplasms

Cytoxan demonstrated therapeutic advantage over certain other agents in a recent 12-month study* of 130 patients, most of whom were refractory to previous treatment:

DISEASE	NUMBER OF PATIENTS	RESULTS				INADEQUATE TRIAL
		GOOD	FAIR	TRANSIENT	FAILURE	
Lymphoma	74	34	3	5	23	9
Hodgkin's Disease	29	10	3	4	9	3
Lymphosarcoma	21	15	0	0	3	3
Multiple Myeloma	16	9	0	0	4	3
Reticulum Cell Disease	8	0	0	1	7	0
Leukemia	23	10	0	0	8	5
Chronic Lymphatic Leukemia	8	4	0	0	3	1
Acute Monoblastic Leukemia	11	5	0	0	3	3
Acute Myeloblastic Leukemia	4	1	0	0	2	1
Carcinoma (Breast, Lung, and Solid Tumors)	29	2	1	1	23	2
Miscellaneous (Mycosis Fungoides, Psoriasis)	4	0	0	1	3	0
Total	130	46	4	7	57	16

Adapted from Wall, R. L., and Conrad, F. G.*

Note that the neoplastic disorders most responsive to Cytoxan were lymphosarcoma, multiple myeloma, Hodgkin's disease, and chronic lymphatic leukemia. Occasionally, good results were observed in acute monocytic leukemia and carcinoma of the breast.

Other advantages noted in this study*

• multiple routes of administration, permitting prolonged maintenance therapy • lack of latency period for bone marrow depression • failure to produce significant thrombocytopenia • potential therapeutic effect in diseases usually unresponsive to other mustard compounds (*e.g.*, myeloma).

*Wall, R. L., and Conrad, F. G.: Arch. Int. Med. *108*:456-482, 1961.

INDICATIONS: Cytoxan is valuable for palliative therapy of certain malignant neoplasms, particularly some of those arising in the reticuloendothelial and hematopoietic systems and certain solid tumors.

Types of cancer which have proved relatively more susceptible or more resistant to Cytoxan therapy may be grouped as follows:

Group I: Neoplasms relatively susceptible to Cytoxan
- Hodgkin's disease
- Lymphomas: lymphosarcoma; giant follicular lymphoma; reticulum cell sarcoma
- Leukemia: acute; chronic
- Mycosis fungoides

Group II: Neoplasms relatively resistant to Cytoxan
- Malignant neoplasms of the breast and the ovary*
- Malignant neoplasms of the lung, the gastrointestinal tract and the genitourinary system, including the cervix and the uterus
- Malignant neoplasms of miscellaneous origin
- Malignant melanomas

*Malignant tumors of these organs are somewhat more susceptible to Cytoxan therapy than are the others included in this group.

DOSAGE: *For neoplasms relatively susceptible to Cytoxan* — Patients with lymphomas and other neoplasms believed to be relatively susceptible to Cytoxan therapy are given an initial dose of 2 to 3 mg./Kg./day intravenously. White blood counts and platelet determinations should be made daily or twice weekly and the dosage adjusted accordingly. Intravenous infusions should be continued for at least 6 days unless otherwise indicated. A leukopenia of between 1500 and 5000 cells per cu. mm. (or lower) may be expected between the tenth and fourteenth day. In the presence of a leukopenia of less than 2000/cu. mm. Cytoxan should be discontinued until the white cell count returns to 2000 to 5000 (usually within a week). Dosage is subsequently adjusted as indicated by the patient's objective response and the leukocyte count. If the patient is subjectively improved, if the size of the tumor has decreased, or if the white cells are satisfactorily maintained between 2000 and 5000/cu. mm. oral dosage may be instituted equivalent to intravenous dosage.

Thrombocytopenia is rarely observed on this regimen. If platelet counts of less than 100,000/cu. mm. are observed, the patient should be watched carefully. If platelets continue to decrease, Cytoxan should be discontinued.

The patient who has had previous treatment with alkylating agents, or x-ray, or is debilitated may be more susceptible to bone marrow depression, and initial Cytoxan doses should be more conservative than the above. Such patients should have more frequent hematologic evaluation. Good medical practice demands access to a reliable hematologic laboratory when using Cytoxan.

For neoplasms relatively resistant to Cytoxan — Patients with carcinomas and other malignant neoplasms believed to be less susceptible to Cytoxan therapy are given a dose of 4 to 8 mg./Kg./day intravenously. Unless there are indications to the contrary, this dose is continued for 6 days, then stopped. Leukopenia usually ensues on the tenth to fourteenth day after the first dose of Cytoxan. Thrombocyte reduction is not common, and platelets may actually increase. The leukocyte count promptly returns toward normal levels in most cases, and as it begins to increase, sufficient Cytoxan is administered to maintain it near 2000 to 5000/cu. mm. This may be accomplished by two intravenous injections weekly, or by oral administration, or by a combination of both routes. An oral dosage of 50 to 200 mg. daily or an intravenous injection of 5 mg./Kg. twice weekly will usually suffice.

The platelet and leukocyte counts should be followed carefully, and the prior treatment history of patients carefully evaluated as delineated above.

Leukopenia as a guide to adequacy of dosage — The best objective measure for dosage seems to be the number of circulating white blood cells. This is used as an index of the activity of the hematopoietic system, especially the bone marrow. The mechanism by which Cytoxan causes a reduction in the level of white blood cells is not known, but cessation of dosage results in an increase in the level, indicating that the hematopoietic system had not been permanently affected. When large doses (8 mg./Kg./day for 6 days) are given initially, the white cell count falls rapidly. Following the cessation of the 6-day course, the white cells may continue to decline for as long as 8 days and then increase. The reduction of the white cell count during Cytoxan therapy and its subsequent increase when therapy is discontinued can be repeated in the same patient. Maximal reduction in leukocyte count indicates the maximal permissible Cytoxan level for therapeutic effect. Leukopenic patients must be watched carefully for evidence of infection.

Total white blood cell and thrombocyte counts should be obtained 2 or more times weekly in order to evaluate therapy and to adjust dosage.

SIDE EFFECTS: Although Cytoxan is related to nitrogen mustard, it has no vesicant effect on tissue. It does not traumatize the vein when injected intravenously, nor does it cause any localized tissue reaction following extravasation. It may be administered intravenously, intramuscularly, intraperitoneally, intrapleurally or directly into the tumor, when indicated. It is apparently active by each of these routes.

Nausea and vomiting are common and depend on dose and on individual susceptibility. However, many investigators accept the nausea and vomiting in favor of maintaining maximal therapy. The vomiting can be controlled with antiemetic agents.

Alopecia is a frequent side reaction to Cytoxan therapy. It has been observed in 28% of the patients studied in this country. The incidence is greater with larger doses. The loss of hair may first be noted about the 21st day of therapy and may proceed to alopecia totalis. This effect is reversed following discontinuance of Cytoxan; during reduced maintenance therapy, hair may reappear. It is essential to advise the patient in advance concerning this effect of the drug.

Dizziness of short duration and of minor degree has occasionally been reported.

Leukopenia is an expected effect and can be used as a guide to therapy. Thrombocytopenia may occur, especially after large doses. The leukocyte or platelet counts of an occasional patient may fall precipitously after even small doses of Cytoxan, as with all alkylating agents. The drug should be discontinued in such patients and reinstituted later at lower dosage after satisfactory hematologic recovery has occurred. Prior treatment with x-ray or with other chemotherapeutic agents frequently causes an earlier or exaggerated leukopenia or thrombocytopenia after Cytoxan medication. Only rarely has there been a report of erythrocyte or hemoglobin reduction.

ADMINISTRATION: Add 5 cc. sterile water (Water for Injection, U.S.P.) to 100 mg. of Cytoxan in the sterile vial (add 10 cc. to 200 mg. vial). Shake, allow to stand until clear, remove with sterile syringe and needle and inject.

The freshly prepared solution of Cytoxan may be administered intravenously, intramuscularly, intraperitoneally, intrapleurally, or directly into the tumor. The solution should be administered promptly after being made but is satisfactory for use for three hours after preparation.

If the patient is receiving a parenteral infusion, the Cytoxan solution may be injected into the rubber tubing if the solution is glucose or saline.

No thrombosis or thrombophlebitis has been reported from injections of Cytoxan. Extravasation of the drug into the subcutaneous tissues does not result in local reactions.

PRECAUTIONS: Cytoxan should not be given to any person with a severe leukopenia, thrombocytopenia, or bone marrow infiltrated with malignant cells. It may be given with suitable precautions to patients who have had recent x-ray treatment, recent treatment with a cytotoxic agent, a surgical procedure within 2 to 3 weeks, or debilitated patients.

AVAILABILITY: Cytoxan is available as follows:

Cytoxan for Injection, 100 mg., a sterile dry-filled vial containing 100 mg. cyclophosphamide and 45 mg. sodium chloride. Packaged, 12 vials per carton.

Cytoxan for Injection, 200 mg., a sterile dry-filled vial containing 200 mg. cyclophosphamide and 90 mg. sodium chloride. Packaged, 12 vials per carton.

Cytoxan Tablets for oral administration, 50 mg., white, round tablets, flecked with blue for easy identification. Packaged, 100 tablets per bottle.

For a copy of the Cytoxan brochure, or other additional information on Cytoxan, communicate directly with the Medical Department, Mead Johnson Laboratories, Evansville 21, Indiana.



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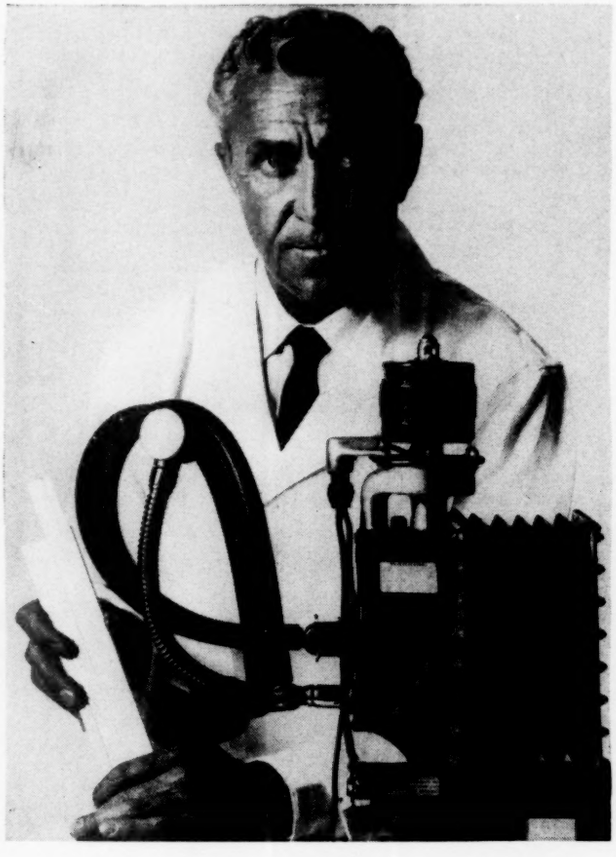
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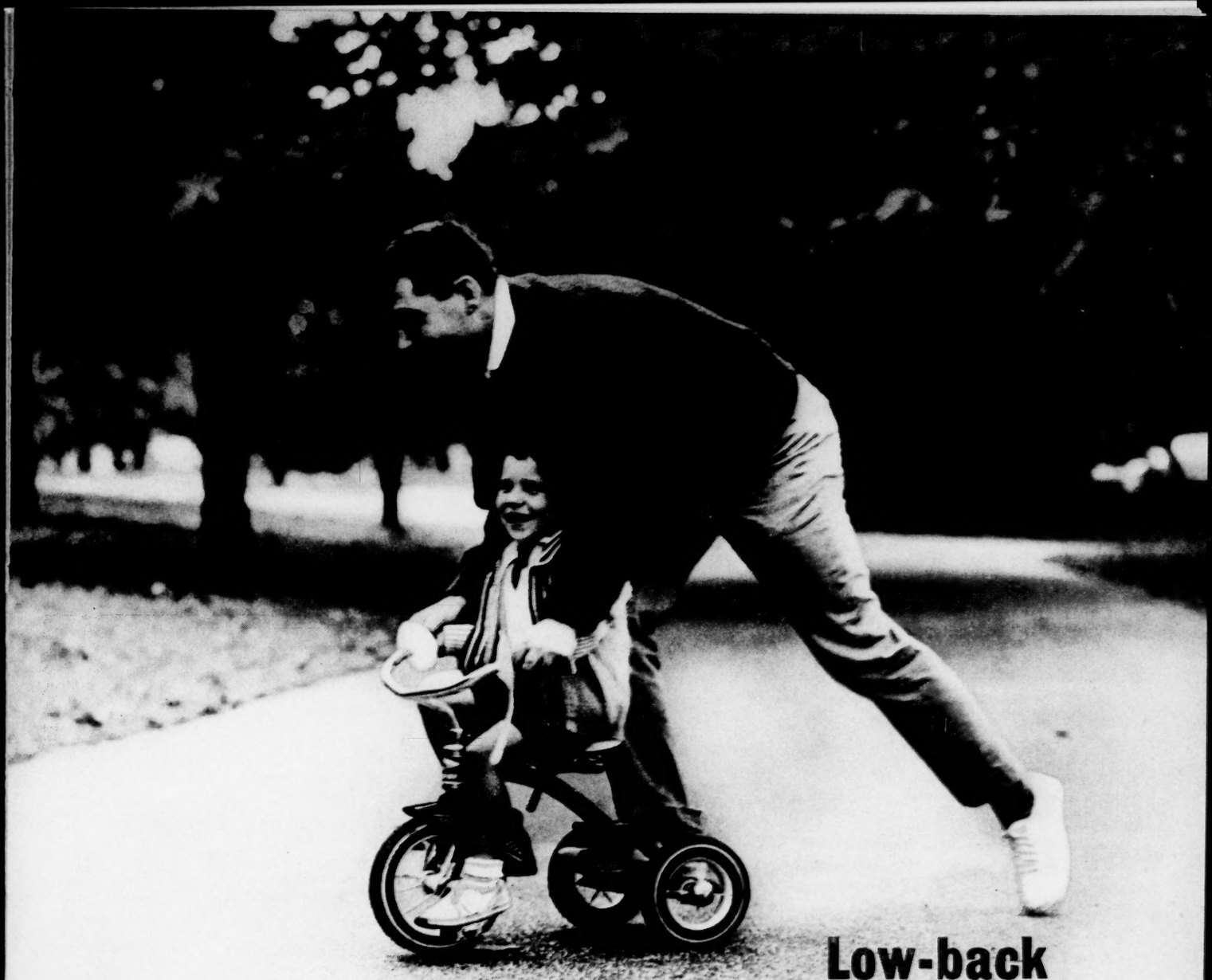
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See page 51 for description, indications, dosage, precautions, side effects, and how supplied.

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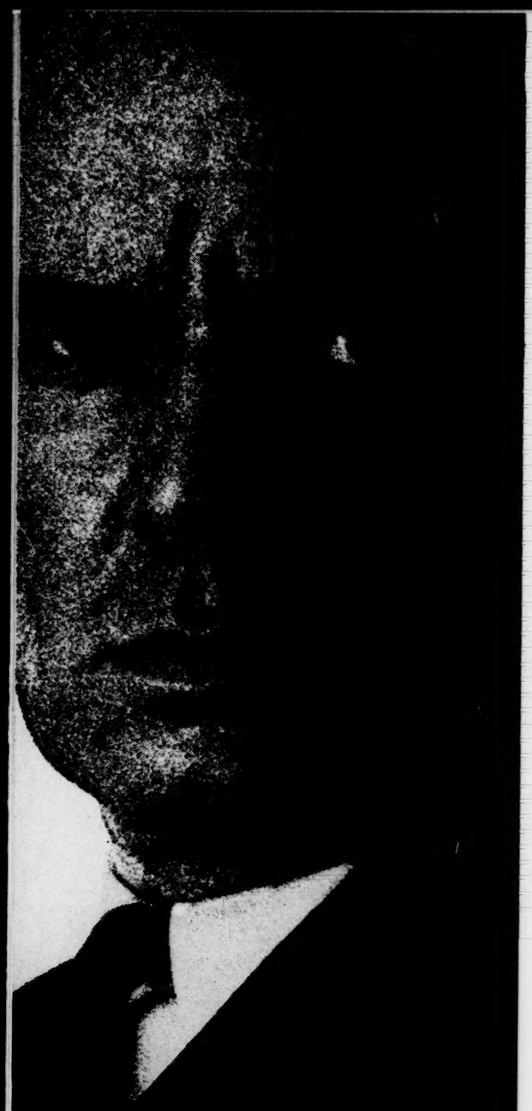
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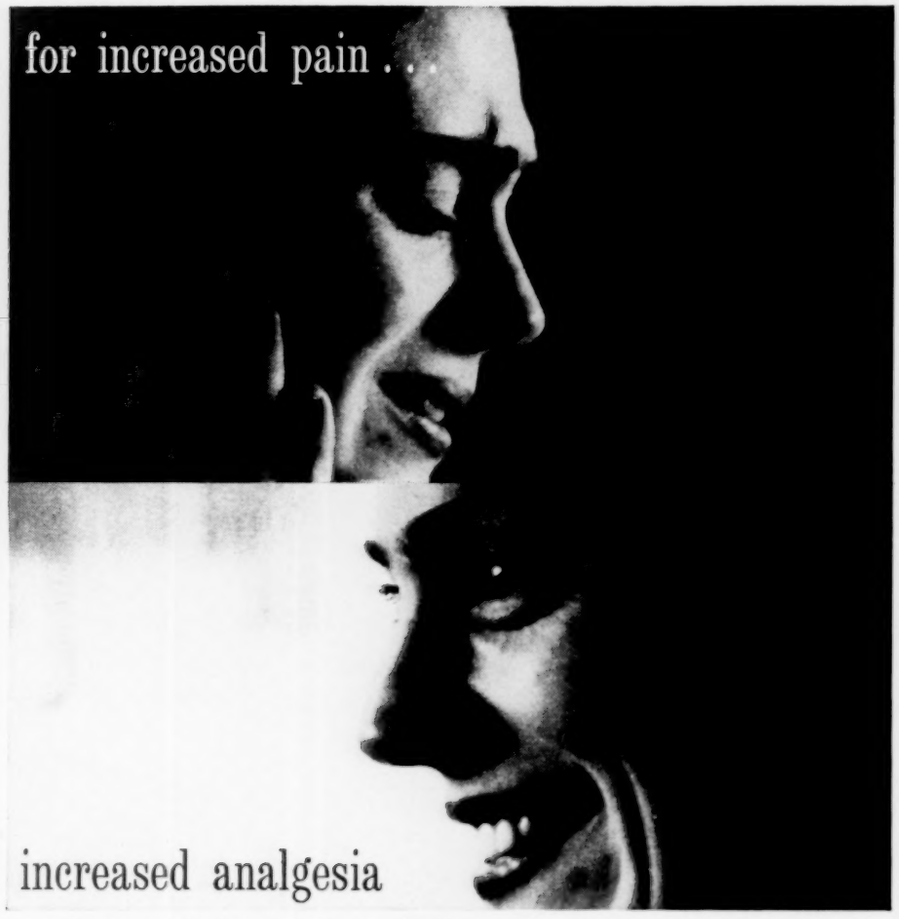
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The American Journal of Medicine

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Symposium on Diabetes

Foreword

EVERY field of medicine has been profoundly affected by the current development of biochemical and biophysical technics and by the revolution in thought which they have induced. This impact of the basic sciences is most clearly demonstrated by diabetes. Understanding the metabolic disturbance of this disease is dependent upon our knowledge of the handling of foodstuffs by the cell and of the chemical regulation of these processes.

As yet we do not possess the insight into the primary genetic defect(s) which prepare(s) the ground for the disorder. It therefore becomes necessary to approach it indirectly. Conn and Fajans review the period which precedes the overt appearance of the classic metabolic deviation in diabetes, hyperglycemia, a period now termed "prediabetes." They consider it a dynamic phase during which endogenous conditions, arising on the basis of the genetic changes, act upon the beta cell apparatus and eventually overcome its resistance. Such studies, coupled with the exact description of the functional changes in the minute morphology of the beta cell brought about by alterations in the environment of the insulin-producing and -secreting tissue, will provide insight into the possible etiologic factors of functional stress and disruption of the beta cell. Paul Lacy's review of the progress in this area, to which he has contributed so much, serves to orient us on this fascinating subject.

Experimental diabetes produced by surgical removal of the pancreas, or by chemical destruction of beta cells, faithfully mimics the diabetic deviations in foodstuff metabolism of the human disease, but it does not reproduce the total picture. Thus, the problems of pregnancy in diabetic subjects, the specific angiopathies and the neuropathies are not yet explored in the animal. Spontaneous diabetes occurs sporadically in domestic pets, and the study of differences between this form and that following pancreatectomy is therefore of great interest. The finding of an animal colony which exhibits a genetic diabetic trait is an event of prime importance. Thus the paper by Meier and the contributions by Yerganian and his associates bear upon the possibility of approaching the full aspect of human diabetes through animal experimentation, including genetic manipulation.

The refinement of technics for the specific quantitative determination of proteins by electrophoretic, chromatographic and labeling technics has led to better understanding of the immunologic behavior of the insulin molecule, the quantitative determination of circulating insulin and the identification of insulin stored in the beta cell. Dr. Berson and Dr. Yallow review these areas in the light of their extensive experience. It is obvious that the ability to determine insulin specifically in human plasma would contribute to our understanding of both the "prediabetic" and the mellituric phases of the disease. Whether the metabolic aberrations are due to absolute or relative lack of insulin, or to inadequate regulation of hormonal output has yet to be determined.

Allied to these considerations is the demonstration by Wright of the production of the effects of insulin lack by the extrapancreatic inactivation of insulin, using specific antibodies

as the effective agents. This work has already shown that diabetic alterations can be produced by a "peripheral" attack on insulin, and that hyperglycemia results within twenty to thirty minutes of insulin binding, thus demonstrating the continuous operation of the hormone.

The papers by Van Itallie and by Levine reflect the changing viewpoints concerning the detailed picture of intermediary metabolism, and the possible points at which hormonal and other integrative regulation is exerted. It becomes evident that cytoarchitectural considerations are bound to play an ever more important role in metabolic control. The primary part played by adipose tissue in regulating the relationship between carbohydrate turnover and fat turnover of the body is again stressed by the most recent work.

It should be emphasized that we lack even an approach to biochemical investigation of the vascular and neurologic aspects of diabetes; and we are still meandering our way into the chemistry of the disease prior to the appearance of hyperglycemia.

The advent of the hypoglycemia-producing sulfonylureas and of the biguanides stimulated much clinical as well as animal investigation in this field. Undoubtedly, one action of the sulfonylureas is activation of insulin release from the beta cell. From a basic standpoint, these compounds may be useful tools for exploring mechanisms of insulin release. On the clinical level, employment of these drugs has reemphasized the differences between the adult and juvenile forms of this disease. The biguanides hold interest because their mode of action is still obscure and seems to be exerted directly on the sugar-consuming tissues. Marble critically examines the range of usefulness of the oral drugs, their indications and the need for careful selection of patients. It would seem that, properly used, the oral antidiabetic agents have been and are continuing to be useful in the therapy of older diabetic patients and of some juvenile patients.

I could point out many areas in the vast field of diabetes which are not covered by this symposium. However, complete coverage was not attempted nor would it be possible within the scope of a journal issue. It was thought that it might be rewarding to examine the progress in those areas which held significant prospects for the near future.

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New York, New York

The Prediabetic State*

A Concept of Dynamic Resistance to a Genetic Diabetogenic Influence

JEROME W. CONN, M.D. *and* STEFAN S. FAJANS, M.D.
Ann Arbor, Michigan

At present nothing is known of the mechanisms which are operative in the future diabetic patient during the years which precede loss of tolerance for carbohydrate. Furthermore, there are no biochemical tests available today that can identify with certainty a person who is believed to be in the prediabetic state. The term "prediabetes" should not be used as a clinical diagnosis. When we can detect an early abnormality which precedes and is not dependent upon insufficient insulin activity, then, and only then, can one justify a clinical diagnosis of prediabetes. When the parameter measured does depend upon diminished activity of insulin even though transient (as in diabetes during pregnancy or infection), or upon diminished functional reserve of the beta cells (as may be indicated by a positive reaction to a cortisone-glucose tolerance test), the situation should be regarded as subclinical diabetes rather than prediabetes. The concept, however, of a prediabetic state has great value because future progress in altering the clinical manifestations of diabetes is dependent upon uncovering the biochemical abberations which characterize the prediabetic state.

Prediabetes, then, means prior to diabetes. It does not mean mild diabetes. People who exhibit such borderline diminution of carbohydrate tolerance that one cannot confidently call them either diabetic or non-diabetic, are not "prediabetic." They are "diabetes suspects" until they give convincing evidence that they are either diabetic or non-diabetic subjects. As will be indicated, this point of view is applicable at the present time to results obtained either with the standard glucose tolerance test or with the cortisone-modified glucose tolerance test.

We believe that most experienced students of clinical diabetes will agree with the following statements: (1) Diabetes mellitus can be defined as an abnormal metabolic state induced by deficient insulin activity. (2) At present, the most sensitive clinical index of the existence of this state is afforded by glucose loading tests. (3) Diabetes is genetically determined, and in whatever way the genetic determinant eventually brings about evidence of deficient insulin activity the basic abnormality has been present since conception. (4) The prediabetic *period* is defined as that period of time from conception to the demonstration of diminished insulin activity by whatever method is considered to be most sensitive in this respect at that time. This means that as more sensitive methods are discovered, the prediabetic period will become shorter. (5) The prediabetic *state* implies as yet undiscovered metabolic derangements which (a) lead eventually to insufficient insulin activity and (b) may give rise also to the generalized angiopathy which characterizes the diabetes syndrome. (6) A breakthrough in knowledge regarding mechanisms at work in the prediabetic state would offer great potential benefits to mankind. The area deserves concentrated research effort.

This paper presents a few data and much speculation about these statements.

The large and increasing diabetic population of the world, together with final general acceptance that diabetes mellitus is a genetically transmitted disease, has led to renewed interest in finding means of early recognition of its existence. It has been but a few years since clinicians insisted that a diagnosis of diabetes mellitus was not justified unless continuous hyperglycemia and glycosuria could be demon-

* From the Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan. This study was supported in part by Research Grant A-888 from the National Institute of Arthritis and Metabolic Diseases Division, U. S. Public Health Service, Bethesda, Maryland. Manuscript received August 30, 1961.

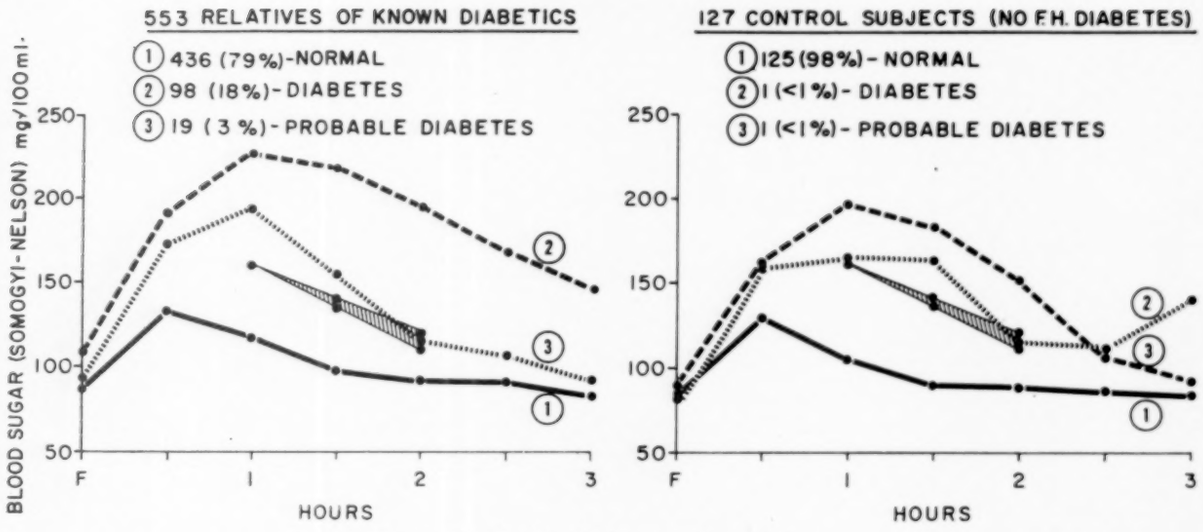


FIG. 1. Standard oral glucose tolerance tests.

strated. Presently, all experts agree that a diagnosis of diabetes mellitus can be made in a completely asymptomatic patient on the basis of the results of a carefully performed glucose tolerance test. Although it is still difficult to obtain agreement among clinical investigators as to what constitutes the *smallest* impairment of carbohydrate tolerance that can justify a confident opinion that diabetes mellitus definitely exists, the area of disagreement has narrowed considerably. For example, when a "true" blood sugar method is used, such as that of Somogyi-Nelson, all will agree that diabetes mellitus exists when the following glucose tolerance curve is obtained: fasting 80 mg./100 ml., one hour 160 mg./100 ml., one and a half hours 150 mg./100 ml. and two hours 140 mg./100 ml., providing, of course, that one has ruled out such other reversible causes for loss of carbohydrate tolerance as antecedent carbohydrate restriction. Cushing's syndrome, acromegaly, pheochromocytoma, liver disease and the like. Based upon an extensive experience with a standardized glucose tolerance test over a long period of time we believe that diabetes mellitus exists when a glucose tolerance curve falls at or above the following: one hour 160 mg./100 ml., one and a half hours 140 mg./100 ml. and two hours 120 mg./100 ml. While most workers agree that the two hour value can be lowered from 140 to 120 to include in the diabetic group a large number of patients formerly designated as "potential" diabetic subjects, one begins to meet resistance to these criteria as the two hour value approaches 120 mg./100 ml. We recognize that as one lowers the two hour criterion level closer and closer toward normal (we regard 110 or below at two hours to represent normal carbohydrate tolerance providing that the one hour level and the one and a half hour levels are also below 160 and 135 mg./100 ml., respectively) the possibility for overlapping between normal subjects and diabetic subjects will increase rapidly at some critical point. Our data indicate that at a two hour criterion level of 120 mg./100 ml. the point at which significant overlapping begins is still some distance away.

When a carefully standardized glucose tolerance test was employed (three day high carbohydrate preparation, Somogyi-Nelson blood sugar method and the diagnostic criteria already outlined) in 127 subjects without a family history of diabetes or large babies, we obtained only one glucose tolerance test result indicative of diabetes and another one indicating probable diabetes. (Fig. 1.) On the other hand, when the same testing procedure was applied to 533 healthy close relatives (parents, siblings and children) of known diabetic patients 436 (79 per cent) had normal results of glucose tolerance tests. Ninety-eight subjects (18 per cent) were found to have diabetes. Curve 2 is the composite curve for these subjects who were previously not suspected of having diabetes and in whom no diagnosis of diabetes had been made. They ranged in age from eleven to sixty-six years, with a mean of 37.5 years. Nineteen of the 533 sub-

jects (3 per cent) gave curves indicating "probable diabetes" or "diabetes-suspect" (curve 3). While one should not expect infallability of any test which must be interpreted on the basis of arbitrary criteria, the experience shown in Figure 1 provides confidence that these criteria constitute a sensitive indicator of the presence of diabetes. We consider it significant that use of this standardized testing procedure, together with the criteria set forth for interpretation of results, indicates that 18 per cent of close relatives of diabetic patients already have unsuspected but detectable diabetes; and that when the same technics are applied to a large group of subjects with no known familial diabetes less than 1 per cent are found to have previously unrecognized diabetes. We are, of course, aware of the report of Unger [*1*] that 25 per cent of people in a general population group have mild loss of carbohydrate tolerance. Such a conclusion cannot be confirmed by our data but the results are not comparable since the testing procedure as well as the interpretative criteria are different in the two studies.

Periodic re-evaluation of patients with mild abnormalities of glucose tolerance over a prolonged period of time is of major importance in justifying the validity of our criteria for the presence of diabetes mellitus. Further impairment of carbohydrate tolerance, clearly diagnostic of diabetes, occurs frequently, as we [*2*] and other investigators [*3*] have reported. Data to be shown presently suggest that the standardized glucose tolerance test still remains the most sensitive current test for the detection of latent, asymptomatic, unrecognized, mild diabetes mellitus.

Contemporary thought makes it mandatory that we detect the patient with latent diabetes and treat him since it is hoped that by so doing diabetic angiopathy may be prevented or at least delayed. There is a growing conviction, however, that by the time diabetes mellitus becomes evident, the vasculature has already suffered an insult which will manifest itself in later years. It is probably true that we are dealing with a damaged person by the time we are able to make the diagnosis of mild diabetes, but we need not assume that he is irreversibly damaged at birth.

It is our conception that the earliest loss of carbohydrate tolerance represents a late stage in the total syndrome which eventuates in the development, among other things, of hyperglycemia and glycosuria. For want of a good term, we would describe the syndrome temporarily as a genetically transmitted abnormality consisting of an elusive biochemical aberration which *may* eventually disclose its presence by evidence of diminished insulin activity. It is likely that the genetic aberration affects many tissues of the body and is at work from the time of birth, or even before.

In order to provide a matrix upon which to speculate further, one might visualize the genetic defect (? enzymatic) as one which affects some aspect of polypeptide synthesis (insulogenesis, vascular structural fabric, nervous tissue structure, others) and also as one which can be present in various degrees of severity. Biogenesis of insulin is imperfect. The beta cells fabricate "active" and "inactive" insulin, the proportion of each being dependent upon the intensity of defective mechanism. A compensatory increase in beta cell function occurs perhaps through a feed-back system controlled by the level of "active" insulin and stimulated by a pituitary insulotropic hormone (? growth hormone). Thus a compensatory mechanism is invoked which is based originally upon the need for more insulin. This mechanism (secretion of an increased amount of pituitary insulotropic hormone [*4*]), although it is a secondary one, carries with it the potentiality of eventually driving the insular system to destruction. In terms of pathogenesis this point could be crucial since the pituitary diabetogenic influence would not be the primary, genetically transmitted aberration but merely the normal response to hypoinsulinism. This thesis is not inconsistent with the findings of Vallance-Owen et al. [*5*] of (1) greater than normal quantities of a serum albumen-bound insulin antagonist in obese diabetic patients as well as in "prediabetic" patients (subclinical diabetic patients by our definition) and (2) supernormal quantities of a similar substance in the blood of depancreatized cats but not of depancreatized-hypophysectomized cats [*6*]. It is clear that presence of the antagonist in increased amounts in depancreatized animals is a response to insulin deficiency. It is likely that its presence in diabetic patients is also the result of insulin insufficiency rather than the cause of it.

Thus the insular system would be under a heavy burden from the time that it first begins to function. In order to produce enough "active" insulin, a given quantity of inactive polypeptide also is secreted. The young insular system, hav-

ing enormous reserve functional capacity, manages for a variable length of time. Eventual beta cell failure will then be determined by the combined influences of the genetic and superimposed environmental factors.

If some such mechanism as that postulated should exist, the prediabetic state could indeed be regarded as one of dynamic resistance to a diabetogenic influence. Under these circumstances the earliest loss of tolerance for carbohydrate would be not only the point where pancreatic beta cell function begins to decompensate but also the point of maximal effort of which these cells are still capable without rest. Reserve functional capacity has been lost. From such a point of view one can consider that the genetic diabetogenic element of this broad disease complex may exist in severe, moderate or mild form; that when it is severe, betacytotropic pressure is great and gives rise to early acute beta cell exhaustion and the so-called growth-onset type of diabetes. When the diabetogenic facet is moderate in intensity, beta cell decompensation is delayed. Because of chronicity and adaptation, beta cell failure may not be complete. This could produce the maturity-onset type of diabetes. When the diabetogenic element is mild it may never expose itself as a defect in carbohydrate metabolism since beta cell compensation may be complete and may be maintained indefinitely. Under such circumstances other manifestations frequently observed in diabetic patients may appear in the absence of a demonstrable defect in the metabolism of carbohydrate. On the basis of such postulations diabetes mellitus, as we are able to diagnose it by measuring decreased tolerance for carbohydrate, would be a late manifestation of a widespread process which has not been dormant but on the contrary extremely active, and has finally outlasted the capacity of the beta cells to respond further. Certainly we must seek to study parameters other than changes in carbohydrate metabolism which manifest themselves so late; parameters other than those designed to measure the effects of diminished insulin activity.

While needing and seeking other means of recognizing the dynamic diabetogenic period which long precedes the earliest decrease of carbohydrate tolerance, present knowledge limits us, practically, to methods which measure the response of the blood sugar to glucose loading. Recently a new test, the sodium tolbutamide response test, has been proposed as a means of recognizing mild cases of diabetes mellitus [7]. Could this help us to move a little further back into the prediabetic period? Unger and Madison [7] have reported that this test separates correctly mildly diabetic patients from non-diabetic patients in approximately 95 per cent of the cases. It is noteworthy, however, that the diabetic subjects in their series were by no means borderline. All had glucose tolerance test results in which the highest blood glucose level exceeded 200 mg./100 ml., the two hour level exceeded 170 mg./100 ml. and the three hour level exceeded 130 mg./100 ml.

We have compared the sensitivity of the intravenous sodium tolbutamide response test and the standard oral glucose tolerance test by performing both procedures in mildly diabetic patients who manifested various degrees of mild loss of carbohydrate tolerance [8]. Of the two testing procedures, each performed sixty times in a total of forty-nine patients, the standard oral glucose tolerance test was found to be far more sensitive for the detection of mild diabetes. On the left side of Table I the results of the glucose tolerance tests have been classified into five groups according to the degree of abnormality. The results of the tolbutamide response tests have been interpreted by the criteria of Unger and Madison [7] as abnormal, presumptively abnormal and normal. Of six patients with relatively mild diabetes whose fasting blood sugar levels were above 120 mg./100 ml. (Somogyi-Nelson method), clearly diagnostic of the disease (Class I), five had abnormal and one had a presumptively abnormal response to the tolbutamide test. In two of these same patients (line 2 of class I) carbohydrate tolerance improved with therapy to that designated as class IV. This, of course, is still clearly a diabetic response by glucose tolerance test criteria. However, the response of these patients to the tolbutamide tests had now become normal. It is evident, therefore, that the glucose tolerance test is a more sensitive indicator of the presence of mild diabetes than is the tolbutamide response test.

Of three patients with fasting blood sugar levels between 110 and 119 mg./100 ml. and grossly abnormal reactions to glucose tolerance tests (class II), one had a normal reaction to the tolbutamide test. Fourteen patients were grouped in class III since they had fasting blood sugar levels below 110 mg./100 ml. but one hour blood sugar levels over 200 mg./100 ml. and two hour

TABLE I

COMPARISON OF GLUCOSE TOLERANCE TEST AND TOLBUTAMIDE RESPONSE TEST IN AMBULATORY SUBJECTS WITH STRONG FAMILY HISTORY OF DIABETES MELLITUS

Class	Glucose Tolerance Test		Tolbutamide Response Test			
	Blood Sugar (mg./100 ml.)	After Improvement to Class	No. of Tests	Abnormal	Presumptively Abnormal	Normal
I	FBS >120	...	6	5	1	...
		IV	2*	...	...	2*
II	FBS 110–119 Abnormal GTT	...	3	2	...	1 (33%)
III	FBS <110	...	14*	8	2*	4 (29%)
	GTT 1 hr. >200	IV	4*	...	1	3*
	GTT 1 hr. >200	V	3	...	1	2
	GTT 2 hr. >180	VI	1	...	..	1
IV	GTT 1 hr. >180	...	13	5	2	6 (46%)
	GTT 2 hr. 140–180	V	1	...	1	...
V	GTT 1 hr. >160	...	13	9	2	2 (15%)
	GTT 2 hr. 120–140					
Total	...	...	60	29	10	21 (35%)

* Including patient with subtotal pancreatectomy.

levels over 180 mg./100 ml. during the glucose tolerance test. Four of the fourteen patients had normal responses to the tolbutamide tests. In eight of these patients glucose tolerance improved with treatment to class IV, V or VI. In six of the eight patients the response to the tolbutamide test returned to normal. Six of thirteen patients with class IV abnormality had normal reactions to the tolbutamide tests as did two of thirteen patients in class V. Thus of these five groups of patients with mild diabetes 35 per cent had a normal response to tolbutamide tests.

Figure 2 shows an example of the inconsistency of the tolbutamide response test. In this diabetic subject the results of all three glucose tolerance tests indicated the presence of the disease. On the other hand, two of the three tolbutamide tests gave the same results that are found in normal people. The results of this comparison of testing procedures do not imply that from a research point of view important information cannot be obtained by intensive study of a standard tolbutamide response test. They do mean, however, that for clinical diagnostic purposes, and despite the fact that it is more cumbersome, the standardized glucose tolerance test is much more sensitive and dependable for the detection of mild diabetes. It is also clear that the tolbutamide test cannot move us into an earlier portion of the "prediabetic" period.

With an increasing conviction that the earliest loss of carbohydrate tolerance really represents the beginning of the end of a hitherto successful battle to *resist* the development of diabetes, we have made an attempt to evaluate any kind of stigma which could be said to occur more frequently in people whose tolerance for carbohydrate at present is normal but who later would lose tolerance for carbohydrate. Probably the most intriguing phenomenon, one that can be shown in some cases to antedate by many years any demonstrable loss of carbohydrate tolerance, is the occurrence of one or more of the following: repeated spontaneous

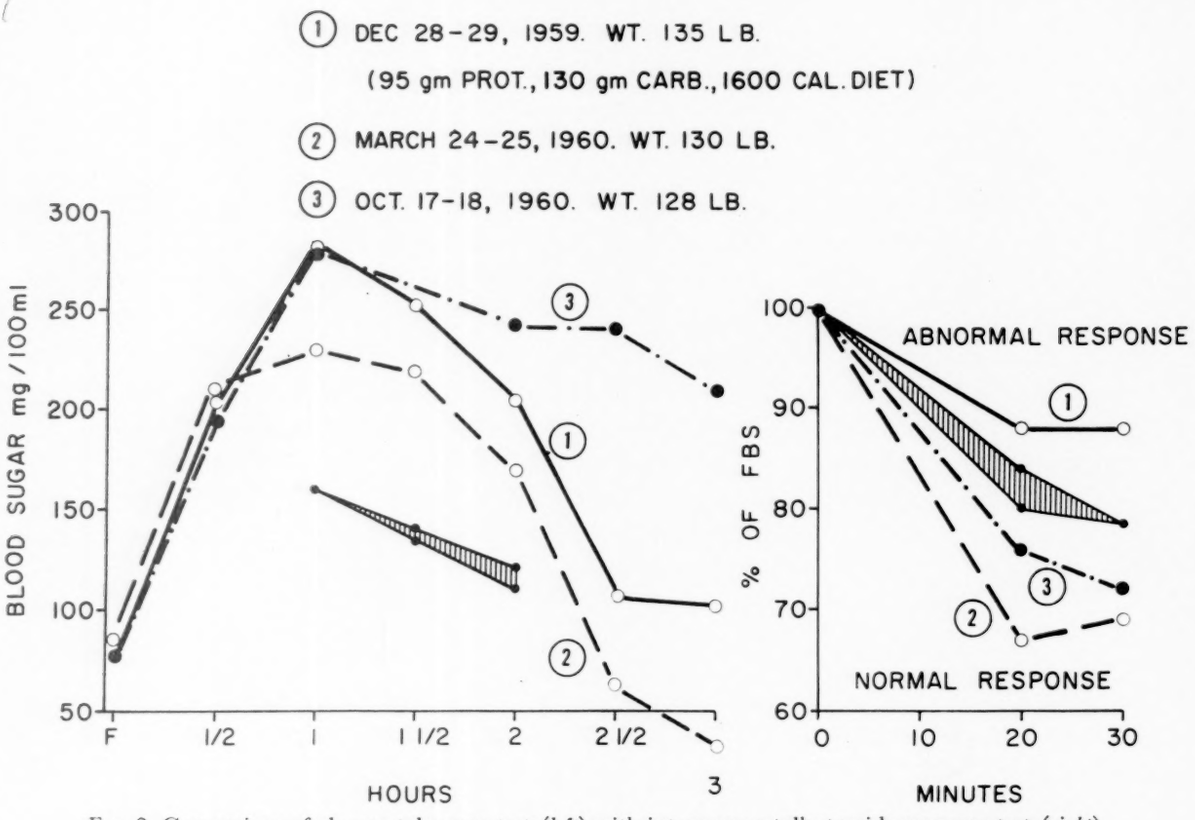


FIG. 2. Comparison of glucose tolerance test (*left*) with intravenous tolbutamide response test (*right*).

abortions, intrauterine deaths, excessively large babies, stillborn infants, neonatal deaths and presence of islet cell hypertrophy in dead infants. It has been well established, of course, that women with frank clinical diabetes have a high incidence of these abnormalities. It is certain that many reports suggesting that women in the "prediabetic state" are almost as prone to these complications of pregnancy as are overt diabetic women are open to question. Had a glucose tolerance test been performed during the so-called "prediabetic" years many such persons would have given a response diagnostic of mild diabetes. Others would have shown "borderline curves" while carbohydrate tolerance in some would not have been detectably abnormal. Obviously, the dividing line between prediabetes and latent diabetes can be established only on a biochemical basis and is dependent upon the criteria that one uses to establish the presence and absence of the diabetic state. Nevertheless, there is sufficient evidence now to indicate that these complications do occur more frequently, despite normal carbohydrate tolerance, in women in whom overt diabetes will subsequently develop [*9*]. It would therefore appear that neither lack of insulin nor the presence of hyperglycemia can be incriminated for the high incidence of fetal abnormalities and perinatal deaths which characterize pregnancies of diabetic and prediabetic women. Nevertheless, such complications should arouse suspicion that either diabetes mellitus or the diabetic diathesis exists. Some investigators [*10–12*] advise that even in the absence of demonstrable diabetes the prior occurrence of such complications of pregnancy warrants the same therapeutic measures in subsequent pregnancies as those that would be applied were the patient clearly diabetic. Results of such procedures are still inconclusive. But if administration of insulin, for example, should prove to suppress such manifestations of the prediabetic state, it would favor the view that a mechanism *compensating* for relative hypoinsulinism is responsible for their appearance.

Retrospective study indicates that frank clinical diabetes is virtually certain to develop in an apparently healthy non-diabetic young woman who gives birth to a 12 pound child

if she lives for another twenty-five years. This gives clear evidence that the prediabetic state is not to be regarded as an inactive, quiescent period, or the "lull before the storm." Rather, it must be viewed as a dynamic period in the evolution of clinical diabetes; a period in which compensatory mechanisms are successfully coping with rampant diabetogenic forces. What metabolic aberration exists at age twenty-five to produce a large baby while carbohydrate tolerance of the mother remains intact for another ten to twenty years? Further, what goes on in the fetal islets of this child to make them hyperplastic while the blood sugar of the mother remains normal? We conceive of this phase of the evolution of the diabetic syndrome as the stage of resistance to the breakdown of carbohydrate metabolism; a period during which the body adapts successfully to continuing assault by a diabetogenic factor. It is this that must be found if we are to apply sound therapy for the prediabetic state. Diabetogenicity probably represents but one of its deleterious potentialities. Treatment with insulin should be expected to correct only one facet of the larger disturbance, i.e., protection of the insular system against the ravages of compensatory hyperfunction and perhaps protection from other effects mediated by the betacytotropic influence. Such a concept is consistent with the clinical characteristics of what can be termed broadly the diabetic syndrome, including manifestations of the diabetogenic period as well as those of the diabetic period.

Thus we find that certain fetal complications of pregnancy, which occur both in frank clinical diabetes as well as before evidence of hypoinsulinism is detectable, can be used as evidence of the existence of the diabetic trait. Important as this may be from a theoretic point of view, its helpfulness in the early recognition of a large number of persons with diabetes obviously is limited. Inasmuch as our aim has been to detect the diabetic trait earlier, and since we believe that detectable loss of carbohydrate tolerance by standard means represents a late stage of the diabetes syndrome, we have directed our efforts for many years towards possible detection while carbohydrate tolerance is still intact. We have now had a long experience with the cortisone-glucose tolerance test as a possible means for earlier detection of diabetes [*13–15*] and we shall present our most recent analysis of the results [*8*].

It bears repetition at this point that any test,

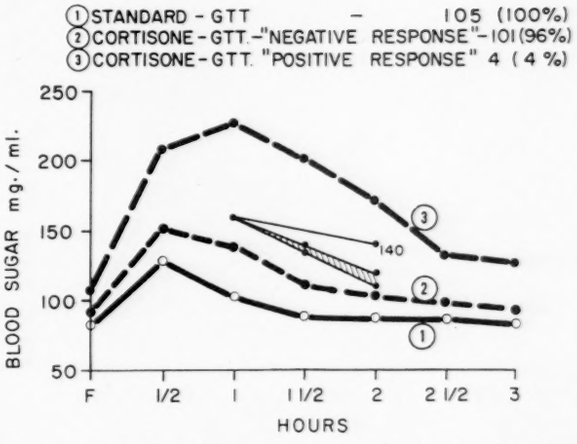


FIG. 3. Cortisone-glucose tolerance tests in 105 healthy subjects with no family history of diabetes or large babies.

regardless of how modified, which depends upon the measurement of decreased carbohydrate tolerance gives no information about the prediabetic state, although it may carry us toward it temporally.

The technic employed for carrying out the cortisone-glucose tolerance test is the same as that previously described [*13–15*]. A blood sugar level of 140 mg./100 ml. at two hours is used as the critical level for the interpretation of the cortisone-glucose tolerance test. (Fig. 3.) A test giving a two hour blood sugar level of 140 mg./100 ml. or above represents a "positive response" while a curve with a level below 140 mg./100 ml. at two hours is regarded as a "negative response." Figure 3 illustrates our findings with the cortisone-glucose tolerance test in a group of 105 control subjects without a family history of diabetes or large babies. Curve 1 demonstrates the composite standard glucose tolerance test. After administration of cortisone 101 subjects (96 per cent) of the control group gave a "negative response" to the test with a mean two hour blood sugar level of 104 mg./100 ml. (curve 2). Four subjects (4 per cent), gave a "positive response" to this test.

Cortisone-glucose tolerance tests were performed in 393 non-diabetic relatives of persons known to have diabetes. (Fig. 4.) It should be emphasized that all these subjects had results of standard glucose tolerance tests which would be considered normal by everyone regardless of the various criteria employed for the interpretation of the glucose tolerance test. After administration of cortisone, 290 of these 393 subjects (74 per cent) showed a "negative response" with a

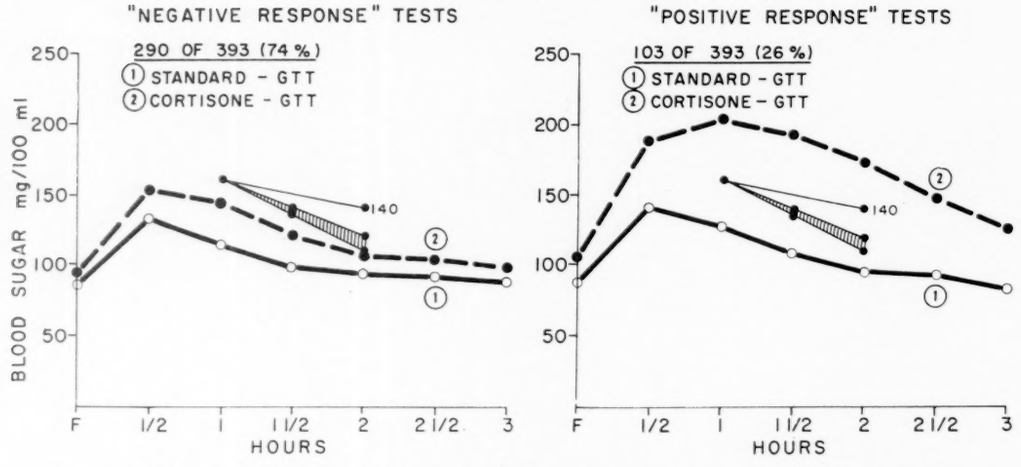


FIG. 4. Cortisone-glucose tolerance tests in 393 non-diabetic relatives of diabetic patients.

mean two hour blood sugar level of 106 mg./100 ml., a response seen in 96 per cent of the control group. Of the 393 subjects 103 (26 per cent), showed a "positive response" as compared with 4 per cent for the control group. Pregnant persons were not included in this series.

Since the cortisone-glucose tolerance test is not a test of genetic susceptibility to diabetes, but merely gives advance notice that insulogenic reserve is diminished (or, expressed differently, that the capacity of the beta cells to respond further is limited), one would expect that young people carrying the diabetic trait should have fewer positive reactions to cortisone-glucose tolerance tests than older ones. The latter group will have arrived at a stage closer to the development of clinical diabetes. Table II compares first the results of the cortisone-glucose tolerance test in the relatives of diabetic patients (all ages) and in the normal control subjects of all ages. Note that the mean age of those with positive responses and those with negative responses is essentially the same in both groups. However, in non-diabetic relatives under the age of eighteen, only 8 per cent gave a positive response to the cortisone-glucose tolerance test while 92 per cent gave a negative response. Conversely, in twenty-five non-diabetic relatives over forty-eight years of age 48 per cent gave a positive response. This high figure among older non-diabetic relatives of diabetic patients is somewhat disconcerting, but it may have important implications. It may indicate only that different criteria for the interpretation of the cortisone-glucose tolerance test may have to be established for the older age group. On the other hand, it may mean that the diabetic trait is much more widespread among relatives of diabetic patients than heretofore believed and that a large number of such persons are able to resist successfully the eventual evolution of clinical diabetes. This would imply that although they maintain normal carbohydrate metabolism the ability of the beta cells to respond to further stimulation is considerably limited. In some of these people, of course, diabetes will develop before they die.

TABLE II

Age Group	Total No.	Positive Response	Negative Response	Age (yr.) Mean	Age (yr.) Range
Cortisone-Glucose Tolerance Test in Non-Diabetic Relatives of Diabetic Subjects					
All ages	393	103 (26%)	...	35.6	12–67
		...	290 (74%)	28.1	7–62
Under 18 yr.	78	6 (8%)	...	15	12–17
		...	72 (92%)	13.2	7–17
Over 48 yr.	25	12 (48%)	...	56	49–67
		...	13 (52%)	53.8	49–62
Cortisone-Glucose Tolerance Test in Normal Control Subjects					
All ages	105	4 (4%)	...	33.8	22–46
		...	101 (96%)	27.2	18–66

In non-diabetic relatives (Table III) between the ages of eighteen and forty-eight, or between the ages of twenty and forty, the incidence of a positive response to the cortisone-glucose tolerance test was 29 per cent and 27 per cent, respectively. Note that the ages of the persons who make up the positive and negative response

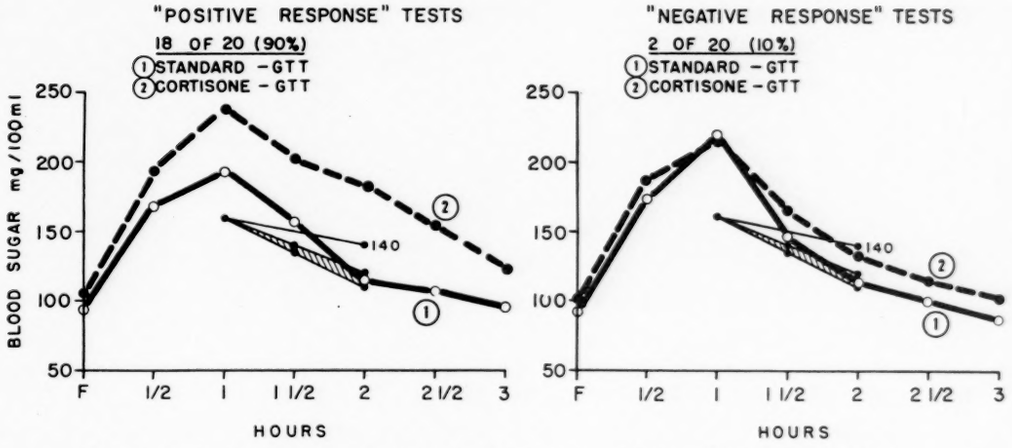


FIG. 5. Cortisone-glucose tolerance tests in twenty subjects with probable diabetes.

groups do not differ greatly from those of the normal control subjects in these same age groups. On the other hand, the percentage incidence of "positive responses" does differ greatly among the relatives of diabetic subjects (27 per cent to 29 per cent) and the control subjects (3 per cent to 4 per cent).

Figure 5 shows the data obtained in twenty subjects classified by their initial reaction to the glucose tolerance test as persons with "probable diabetes" (curve 1). Eighteen of the twenty (90 per cent) gave a "positive response" to the cortisone-glucose tolerance test. They ranged in age between eighteen and sixty-three years (four over forty-eight years) with a mean age of forty years. The two subjects with negative responses were both thirty-nine years old.

Twenty-nine obese patients with mild diabetes were treated with low caloric diets. (Fig. 6.) After weight reduction all subjects exhibited normal standard glucose tolerance curves. Cortisone-glucose tolerance tests were then performed. Twenty-five of the twenty-nine (86 per cent) gave a "positive response" to the test.

In correlating these results it seems significant that about 28 per cent of the group of non-diabetic relatives of diabetic patients respond to this test the same way as do 90 per cent of the subjects with probable diabetes and 86 per cent of the group of obese diabetic patients whose carbohydrate tolerance had returned to normal after reduction of body weight. However, only 4 per cent of the control group without a family history of diabetes gave a positive response to the cortisone-glucose tolerance test.

Time and continued study are needed to determine the true incidence of future diabetes in the "positive response" groups. So far we have followed 128 non-diabetic relatives of diabetic patients over the last seven years. (Table IV.) Of

TABLE III

Age Group	Total No.	Positive Response	Negative Response	Age (yr.) Mean	Age (yr.) Range
Cortisone-Glucose Tolerance Tests in Non-Diabetic Relatives of Diabetic Subjects					
All ages	393	103 (26%)	...	35.6	12–67
		...	290 (74%)	28.1	7–62
18–48	290	85 (29%)	...	34.3	18–48
		...	205 (71%)	32.1	18–48
20–40	223	61 (27%)	...	30.5	20–40
		...	162 (73%)	29.7	20–40
Cortisone-Glucose Tolerance Test in Normal Control Subjects					
18–48	102	4 (4%)	...	33.8	22–46
		...	98 (96%)	27.1	18–48
20–40	93	3 (3%)	...	29.7	22–35
		...	90 (97%)	25.6	20–40

TABLE IV

FOLLOW-UP RESULTS (ONE TO SEVEN YEARS) IN 128 NON-DIABETIC RELATIVES OF DIABETIC PATIENTS

Initial Normal Glucose Tolerance Test		Progression to:		
Cortisone-Glucose Tolerance Test	No. of Subjects	Diabetes (no.)	Probable Diabetes (no.)	Total Abnormal (no.)
Positive response	57	15 (26%)	5 (9%)	20 (35%)
Negative response	71	2 (3%)	0	2 (3%)

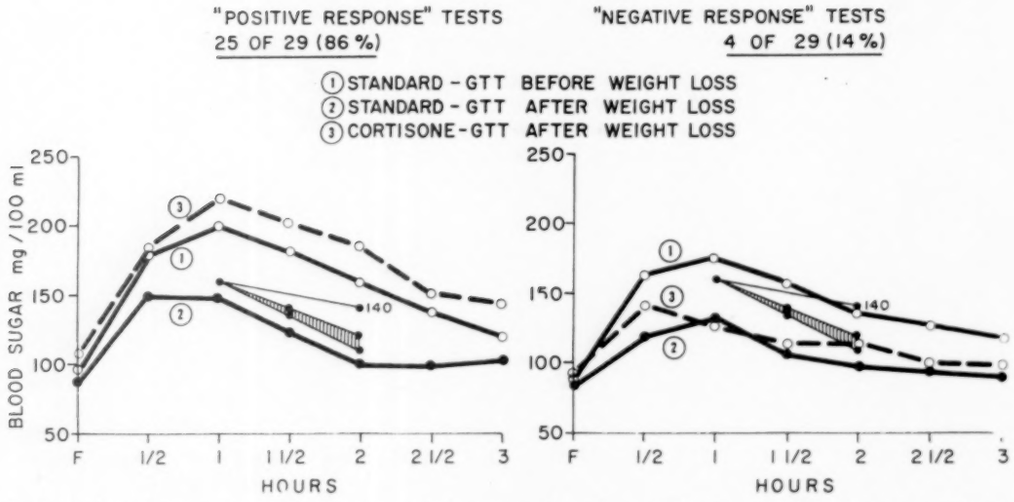


FIG. 6. Cortisone-glucose tolerance tests in twenty-nine obese diabetic subjects after loss of body weight.

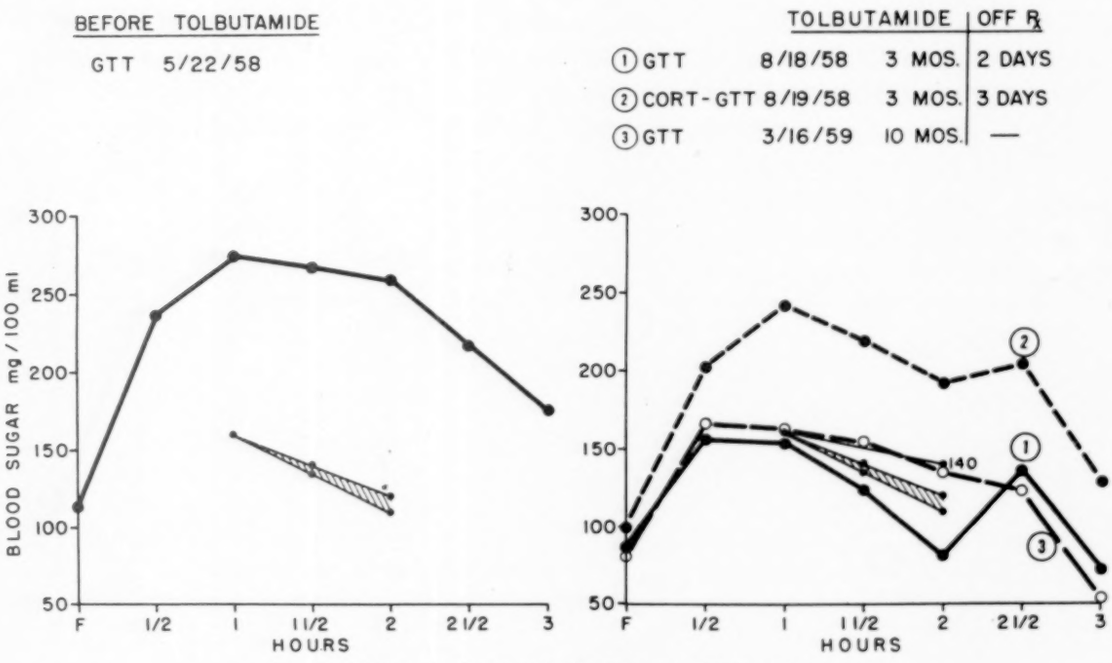


FIG. 7. Effect of tolbutamide therapy on glucose tolerance.

these 128 subjects fifty-seven had had "positive responses" to the initial cortisone-glucose tolerance test. Subsequently diabetes has developed in fifteen (26 per cent) of these fifty-seven subjects, whereas another five (9 per cent) have "probable diabetes." Of the seventy-one subjects with "negative responses" to the cortisone-glucose tolerance test diabetes has developed in only two. To date, then, diabetes or "probable diabetes" has developed in 35 per cent of the group with a "positive response" but in only 2 per cent of the group with a "negative response."

Thus it seems likely that the cortisone-glucose tolerance test may prove to be valuable for earlier detection of the *diabetic* state. Obviously it cannot detect "genetic susceptibility" to diabetes [*15*]. But it probably allows us to pinpoint diabetic patients several years earlier than any other method of testing currently available. This constitutes a small step in the right direc-

tion. However, elucidation of the abnormality which exists in the prediabetic state and which eventuates in hypoinsulinism and generalized angiopathy should be our major goal. We must regard as possible the proposition that the genetically transmitted abnormality might be susceptible in the prediabetic period to exogenous influences; and that such influences might alter or even prevent the clinical manifestations of diabetes as we know them today. In this connection, but not necessarily related to development of diabetes in man, it is of interest that Mirsky [*16*] has found that the diabetogenic effect of growth hormone in dogs is diminished or prevented by simultaneous administration of tolbutamide. In young, asymptomatic diabetic patients treated with tolbutamide over long periods of time we [*17*] have observed return of normal carbohydrate tolerance in some and marked improvement of tolerance in others. Figure 7 shows an example. We have observed similar improvement in patients whose insular system has been rested by diet, insulin or both. But these factors do not account for the results obtained in the tolbutamide studies. Perhaps an action of tolbutamide makes overworked beta cells better able to function under duress. If so, and if, indeed, the prediabetic state is characterized by a dynamic insular response (with eventual failure) to a diabetogenic influence, tolbutamide given in the prediabetic period might prevent or forestall the emergence of hypoinsulinism.

SUMMARY

We have reviewed current considerations and technics involved in attempts at earlier diagnosis of diabetes mellitus. It is concluded that the standardized glucose tolerance test is the most sensitive diagnostic index. The cortisone-glucose tolerance test must still be regarded as being in the investigative stage, although it appears to be emerging as a test capable of detecting beta cell fatigue earlier than it is detected by the standard glucose tolerance test.

A search for parameters of study other than carbohydrate metabolism which could detect the "diabetic trait" before beta cell decompensation occurs is regarded as the most fertile field for future research in connection with earlier detection and ultimate prevention of the diabetes syndrome. The high incidence of fetal complications of pregnancy in women destined to become diabetic many years later supports the existence of an active metabolic aberration long before the insulogenic mechanism becomes overwhelmed.

A concept of a dynamic diabetogenic period existing from the time of birth until beta cell decompensation occurs is presented. It is likely that ultimately we will divide manifestations of the diabetes syndrome into those which precede and those which follow the onset of hypoinsulinism; and the latter group into those which are and those which are not dependent upon insufficient activity of insulin. Such a concept is consistent with the evolution of the various clinical manifestations of the disease.

Finally, we admit that we have taken full advantage of this opportunity to speculate about many aspects of the "diabetes syndrome," one end stage of which expresses itself as hypoinsulinism.

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Electron Microscopy of the Beta Cell of the Pancreas*

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ELECTRON microscopy can be utilized to study the mechanisms by which beta cells form, store and release insulin, and to visualize simultaneously all the intracellular components of a beta cell as well as to show its relationship to surrounding structures in a variety of species [*1–8*]. It is now also feasible to study the internal structure of a single intracellular component such as a beta granule. Moreover, by microchemical technics [*9*] the activity of certain enzymes in beta cells can be measured [*10*]. The fluorescent-antibody method [*11*] has made it possible to search for immunologic differences of endogenous insulin in different species [*12,13*], and as a result of the development of a sensitive method of insulin assay [*14,15*] it is possible to measure quantitatively the insulin content of dissected islets [*16*]. The present report is concerned mainly with electron microscopic observations on the mechanisms of storage, formation and release of insulin from beta cells, together with studies on the ultrastructure of these cells in diabetes.

IDENTIFICATION OF CELL TYPES

With light microscopy the different types of islet cells can be distinguished on the basis of their tinctorial reaction with specific stains such as aldehyde fuchsin [*17*]. With electron microscopy ultrastructural criteria have to be utilized for the characterization and identification of these cells. These distinguishing ultrastructural features have been established by utilizing serial sections and comparing portions of the same cells observed in a thin section with electron microscopy and in a stained section with light microscopy [*1*]. Some of the general distinguishing features are that beta cells contain a larger amount of ergastoplasm or endoplasmic reticulum and thus appear to have a denser cytoplasm than alpha cells. The beta granules show remarkable species variation in their ultrastructure whereas alpha granules appear round, dense and homogenous in all the different species examined. In islets of the guinea pig, C cells are recognized by their pale cytoplasm containing only a few mitochondria, a small amount of ergastoplasm and no distinct secretory granules. Bencosme and Pease [*6*] have demonstrated that the D cell of the cat pancreas may contain distinct, round, homogeneous granules which are less dense than alpha granules or they may have a pale cytoplasm containing smudged, indistinct granules.

Typical Beta Cell. The general ultrastructural features which are common to beta cells in all the different species of animals, including man, are demonstrated schematically in Figure 1. The beta cells are limited by a continuous plasma membrane and are separated from each other by a small intercellular space which is interrupted focally by a cement substance which apparently holds the cells together. Their nuclei are surrounded by double nuclear membranes and nuclear pores can frequently be observed. The beta granules are enclosed by smooth membranous sacs and usually a small space of variable dimension separates the granule from the wall of its sac. The mitochondria are scattered diffusely throughout the cytoplasm and contain the typical cristae that have been observed in other types of cells. The mitochondria are much smaller than those of acinar cells. The Golgi complex is comprised of smooth membranous sacs that are found usually in a perinuclear position, but do not appear to have any particular polar localization within the cell. The remainder of the cytoplasm contains an

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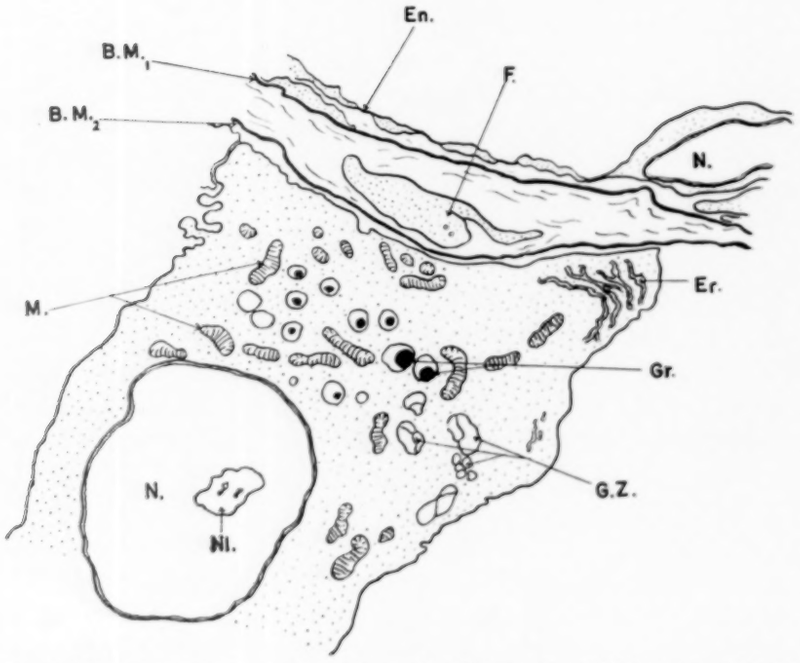


FIG. 1. Normal ultrastructure of a beta cell. The nucleus (N) is enclosed by a double nuclear membrane. A prominent nucleolus (Nl) is frequently observed in the electromicrographs. The beta granules (Gr) are enclosed in smooth membranous sacs. The mitochondria (M) are small and have characteristic cristae within them. The ergastoplasm (Er) is composed of lamellae with attached ribonucleoprotein granules. The Golgi zone is comprised of smooth membranous sacs near the nucleus. The beta cell is separated from the capillary by two basement membranes (BM_1 and BM_2). Frequently cross sections of fibroblasts (F) or nerve fibers are found between the basement membranes. The endothelium (En) has focal areas of attenuations.

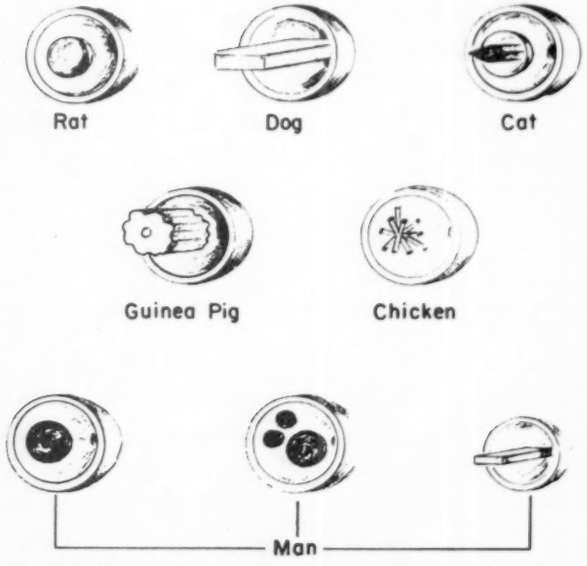


FIG. 2. Representations of the three-dimensional aspect of the ultrastructure of beta granules. These representations are hypothetical at the present time since serial sections and reconstruction of a single beta granule has not yet been accomplished.

abundance of ergastoplasm or endoplasmic reticulum composed, in lamellar arrangement, of two membranes with ribonucleoprotein granules attached to their outer surfaces. Beta cells are separated from capillaries by two distinct basement membranes, one of which is associated with the beta cell, the other with the endothelium. A space of variable dimension is present between these two membranes, and frequently cross sections of fibroblasts or portions of nerve fibers may be observed in this area. The endothelium in the islets is like that of other endocrine glands in that it has focal areas of attenuations in which the cytoplasm has become so diminished that its two plasma membranes are closely apposed and appear as a single line. The endothelium is continuous and does not have any holes or pores passing through it.

Beta granules apparently represent the storage form of insulin, as demonstrated by Hartroft and Wrenshall [*18*]. Before insulin can be secreted into the blood stream it must by some means pass



FIG. 3. Electron micrograph of a normal beta granule of the rat. The granule is round, with a porous internal structure. It is enclosed in a smooth membranous sac. The lamellar ergastoplasm with attached ribonucleoprotein granules is shown in the upper portion of the photograph and mitochondria are present in the lower right portion. Magnification approximately × 60,000.

through the following structures: the encasing membrane sac, the plasma membrane of the beta cell, two basement membranes, a total of three spaces between the beta cell and capillary, two plasma membranes and cytoplasm of the endothelium and finally into the capillary lumen. It is at least theoretically possible that any one of these structures might serve as a potential barrier to the release of insulin in certain forms of diabetes in man.

STORAGE FORM OF INSULIN

Species Difference. Remarkable variation has been found in the ultrastructure of beta granules of different species of animals, so much so that in some instances the species can be recognized by the electron microscopic structure of their beta granules. A diagrammatic representation of the hypothetical three-dimensional structure of beta granules in different species is given in Figure 2.

In the rat, beta granules are round and they appear homogeneous with relatively low powers of magnification; with higher magnification it is apparent that a distinct variation in density is present in the core of the granule. (Fig. 3.) In the dog, beta granules have a rectangular profile in electron micrographs. The three-dimensional appearance of these granules is probably a crystalline plate as shown in Figure 2. In the cat, the central portion of the granule is comprised of a dense prismatic structure which is encased in a less dense homogeneous material. Lever and Jeacock [*19*] have demonstrated that a definite crystalline structure is present in this central prismatic portion of the granule. In the guinea pig, the granules are irregularly shaped and frequently a central clear area is present. In the chicken, the beta granules appear to be composed of sheaves of needle-like crystals. In man, marked variation is present in the ultrastructure of the individual granules; some appear round and one or more may be encased in the same membranous sac, others have rectangular profiles which probably represent cross sections of small crystalline plates.

The striking variation in the ultrastructure of beta granules in different species suggests that the storage form of insulin may be different in these animals. This variation in ultrastructure of the granules may be due to differences in the macromolecular arrangement of insulin within them, differences in the amino acid composition of the insulin or variations in the type of bind-

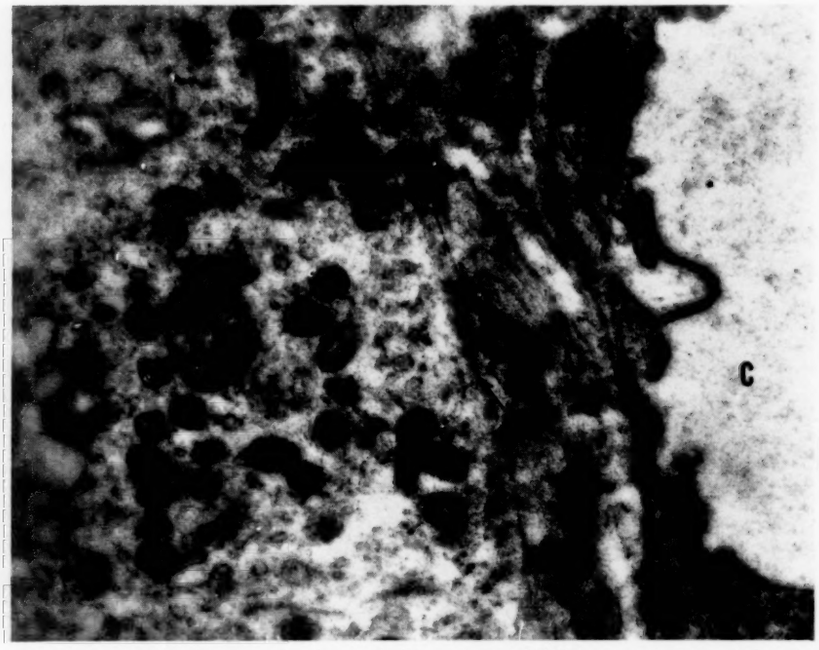


FIG. 4. Electron micrograph of the fibrillar material (F) separating a neoplastic beta cell from the adjacent capillary C. The small fibrils are evident within the neoplastic cell adjacent to its plasma membrane and in the pericapillary space. Magnification approximately × 23,000.

ing protein that may be associated with the stored insulin.

Beta Cell Tumors. In functioning beta cell tumors in man, the neoplastic beta cells release insulin at an uncontrolled rate. The alterations in these cells which render them unresponsive to normal regulatory mechanisms are unknown at the present time. Five beta cell tumors have been studied with electron microscopy [*20*]. In these tumors the beta granules and organelles of the neoplastic cells had the same ultrastructure as normal beta cells in man. The only distinguishing electron microscopic feature observed was the presence of a fine fibrillar substance interposed between the two basement membranes which separate the neoplastic cells and the capillaries. (Fig. 4.) The fibrillar material is not continuous with either basement membrane but appears as a mass of foreign material which has been deposited between them. This substance is apparently comprised of a protein, since its electron microscopic features are similar to the fibrils observed in glial cells, to the tonofibrils of squamous cells and to the perinuclear fibrillar substance observed in normal beta cells of the rabbit. The tiny fibrils are smaller than those of collagen fibers, and they do not have any evidence of cross striations such as would be found in collagen or reticulum. These fibrils may originate within the neoplastic cells. Figure 4 illustrates a neoplastic beta cell containing fibrils close to the plasma membrane and a large mass of similar fibrillar substance between the cell and the capillary. Since this material apparently is a protein substance originating in the neoplastic cells, the possibility was considered that it may represent a fibrillar form of insulin such as has been produced *in vitro* by Waugh [*21*]. We have recently tested this hypothesis by dissecting the fibrillar material from frozen-dried sections of tumor and assaying it for insulin content before and after subjecting it to a reactivation procedure described by Waugh [*22*]. This procedure will convert inactive fibrillar insulin to an active globular form. In these initial studies insulin activity could not be detected in the fibrils, either before or after reactivation. The composition of this fibrillar material originating in the neoplastic cells is unknown at the present time.

The fluorescent antibody technic of Coons and Kaplan [*11*] has been used to demonstrate endogenous insulin in islets of frozen or frozen-dried sections of pancreas from different species [*13*]. The results of these studies in eight different species, including man, indicate that endogenous

insulin of all these species, with the exception of the guinea pig, will react with labeled antibodies to beef insulin. In the initial studies a positive reaction was not demonstrated in islet cells of man obtained at autopsy [*12*]; however, with subsequent improvements in the method and utilization of fresh pancreas obtained at surgery a positive reaction could be demonstrated in human islet cells. The fluorescent antibody method has been applied to five beta cell tumors [*20*]. In each instance a negative reaction was noted in the tumor, whereas a positive reaction was present in the islet cells of the adjacent pancreas and normal human pancreas. This suggests that the endogenous insulin of the tumor may be immunologically different from normal human insulin.

By the epididymal fat pad method of insulin assay it could be demonstrated that insulin was present in these tumors even though it did not react with labelled antibodies. Neoplastic beta cells were dissected from frozen-dried sections of tumor, weighed on a quartz fiber balance and their insulin content determined [*16*]. The insulin content of the neoplastic beta cells varied from six times to a half that present in normal human islet cells, even though it did not react with fluorescent-labeled antibodies to beef insulin. Why islet tumors fail to react immunologically is not known at the present time. Tumor insulin may be bound to another protein within the beta granules in such a way that it cannot react with the antibodies; or the macromolecular configuration of tumor insulin may be different from normal; or the amino acid composition may be different from that of normal human insulin.

MECHANISM OF SECRETION OF INSULIN

Electron microscopic studies of beta cells of the rat pancreas have been made following stimulation either by induction of marked hyperglycemia [*23*] or by administration of tolbutamide [*24*]. The hyperglycemia was produced by repeated injections of large amounts of glucagon and administration of a high carbohydrate diet, as described by Salter et al. [*25*]. In the hyperglycemic animals the earliest electron microscopic change observed was the presence of numerous cytoplasmic processes or microvilli projecting from the surface of the beta cells. Small concavities in the surface of the cell were also present on each side of the microvilli. With continued hyperglycemia the beta cells were almost completely degranulated and their cytoplasm was filled with a lamellar type of ergastoplasm with only a few remaining beta granules. During this later period the number of microvilli decreased. Treatment of rats with tolbutamide produced a similar type of change. Initially, increased numbers of microvilli were present, progressive beta cell degranulation occurred, and the degranulated cells were filled with a lamellar type of ergastoplasm. Release of beta granules and formation of the microvilli apparently occur by the following mechanisms. The beta granules with their surrounding sacs move to the surface of the cell where the membranous sac fuses with the plasma membrane. Subsequently the sacs rupture, the granules are liberated into either the pericapillary or intercellular space where they undergo rapid dissolution, the remnants of their sacs persisting as small concavities at the surface of the cell. This process of secretion is termed emiocytosis. If two granules are liberated close to each other, the cytoplasm between the two remaining concavities appears as a small microvillus projecting from the surface. If additional granules are rapidly liberated at each of these concavities the microvillus protrudes further from the cell as portions of the wall of each sac are added to it. A diagrammatic representation of this method of secretion is shown in Figure 5.

Electron microscopic studies indicate that the mechanism of release of beta granules is the same following stimulation with either hyperglycemia or tolbutamide. Both of these agents probably produce an alteration in intracellular metabolism of the beta cells which results in the migration of beta granules to the cell surface where they are liberated. Future studies of the effect of these agents on the enzymatic activity and metabolism of beta cells should provide further information on the basic mechanism of beta cell secretion. In addition, comparisons of the intracellular metabolic effects of hyperglycemia and tolbutamide may provide a clue to the pathogenesis of certain forms of diabetes in man. In many subjects with maturity-onset diabetes the administration of tolbutamide will apparently liberate sufficient insulin to maintain a normoglycemic state whereas hyperglycemia alone in these subjects is unable to stimulate the release of adequate quantitites of insulin. The results of these electron microscopic studies also suggest that in the rat the disintegration of beta granules occurs extracellularly and ap-

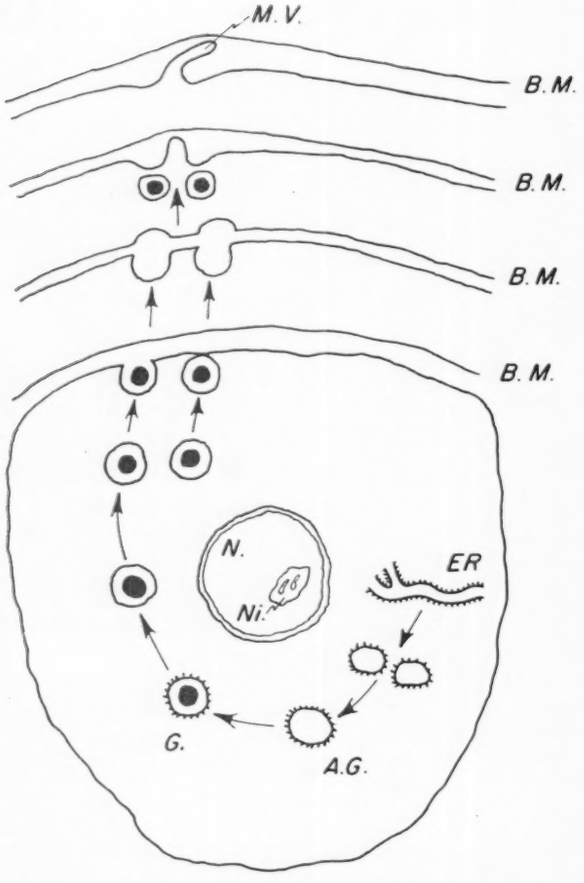


FIG. 5. In the formation of beta granules, the ergastoplasm (ER) is transformed from a lamellar to a saccular form. The ergastoplasmic sacs accumulate an amorphous material within them (AG) which apparently condenses into a distinct beta granule (G). The attached ribonucleoprotein granules are lost and the beta granule is enclosed in a smooth membranous sac. Beta granules are released by ejection from the cell (emiocytosis). Microvilli (MV) are formed as a result of this mode of secretion. BM = basement membrane.

parently does not result from any specific intracellular enzymatic process.

MECHANISM OF INSULIN FORMATION

Electron microscopic studies on beta cells during the regranulation stage following acute injections of tolbutamide have shown that a striking change occurs in the ergastoplasm of these cells [*24*]. Tolbutamide produces rapid degranulation of beta cells. During this stage of degranulation the ergastoplasm of the beta cells has a lamellar structure. When the injections of tolbutamide are stopped, the ergastoplasm becomes transformed into a vesicular or saccular form. These ergastoplasmic sacs have attached to their outer surfaces ribonucleoprotein granules similar to those found on the lamellae of the normal ergastoplasm. The sacs accumulate within them an amorphous material which probably represents a precursor of insulin since the extractable insulin content of the pancreas at this time is extremely low. This amorphous material apparently condenses and forms distinct, round, dense beta granules which are still enclosed by membranous sacs with attached ribonucleoprotein granules remaining on their outer surfaces. Subsequently, the ribonucleoprotein granules are lost and the mature beta granules remain encased in smooth, membranous sacs. Thus in the rat the sequence of events leading from formation to liberation of insulin from beta cells appears to be as follows: (1) ergastoplasm is transformed from a lamellar to a vesicular form, (2) beta granules are formed within the ergastoplasmic sacs, (3) the granules with their encasing sacs move to the surface of the cell where they are liberated by emiocytosis, and (4) in the extracellular space, the granules undergo dissolution and insulin passes across the two basement membranes, through the endothelium and into the blood passing through the islets. These events are depicted diagrammatically in Figure 5.

ULTRASTRUCTURE OF BETA CELLS IN DIABETES

Cytotoxic Destruction of Beta Cells. Electron microscopic studies have been made on the islet cells of alloxan-diabetic rats and rabbits [*26,27*]. In the rat, the earliest change observed in beta cells was vacuolation of the ergastoplasm. Subsequently the mitochondria also became vacuolated, the ergastoplasmic vacuoles increased in size, and the plasma and nuclear membranes disintegrated. As the beta cells disintegrated, macrophages appeared within the islets and phagocytized the necrotic remnants of these cells. In the rabbit, a striking increase in the number of beta granules was observed in the first five minutes after the injection of alloxan. Subsequently the granules disappeared and the beta cells disintegrated in a manner similar to that observed in the rat. In both species the specificity of the cytotoxic effect of alloxan for beta cells was demonstrated. Normal alpha cells with abundant alpha granules were observed adjacent to beta cells that were undergoing necrosis. The electron microscopic studies do not indicate a specific site of action of alloxan on beta cells. The ultrastructural changes observed



FIG. 6. Electron micrograph of massive accumulation of glycogen (G) in a beta cell of a spontaneously diabetic Chinese hamster. A few mitochondria and a small amount of ergastoplasm are present adjacent to the nucleus and at the peripheral margin of the cell. A small portion of an alpha cell containing numerous alpha granules and no glycogen is shown in the lower left portion of the photograph. Magnification approximately × 8,000.

in the ergastoplasm and in the beta granules probably reflect the toxic effect of alloxan on intracellular enzymes of the beta cells.

Glycogen Accumulation in Beta Cells. Weichselbaum and Stangl [*28*] first described vacuolation of beta cells in pancreases from comatose diabetic patients. The vacuolation of beta cells was believed for many years to be due to an accumulation of fluid within them and was therefore called hydropic degeneration. This concept persisted until the studies of Toreson [*29*], who demonstrated that the vacuolation of the beta cells was due to the accumulation of glycogen within them. Electron microscopic studies [*30*] have been made of glycogen accumulation in beta cells of cats which received repeated intraperitoneal injections of glucose by the method described by Dohan and Lukens [*31*]. Serial electron microscopic studies of the pancreases from these cats indicate that glycogen first appears diffusely throughout the beta cells. At later intervals discrete glycogen deposits are evident, displacing the surrounding organelles. Degranulation of beta cells is apparent but no ultrastructural changes are present in the mitochondria, Golgi apparatus or ergastoplasm. Electron microscopic studies of beta cells with more massive accumulations of glycogen have been made in partially pancreatectomized cats treated with growth hormone and in the spontaneously diabetic Chinese hamster [*32*]. In these instances the glycogen almost completely displaces the normal organelles, and the nucleus appears encased in a mass of glycogen. (Fig. 6.) The mitochondria and ergastoplasm remaining in these glycogen-infiltrated cells appear to be normal. Such massive accumulation of glycogen would interfere with the normal secretory activity of the beta cell since it would diminish the amount of ergastoplasm, with a resultant reduction in the ability of the cells to form insulin. The glycogen accumulation apparently does not produce degenerative changes in beta cells since their organelles appear normal although reduced in amount. When insulin is administered to these animals the blood sugar returns to normal and glycogen is rapidly lost from the beta cells. After normoglycemia has been established the beta cells again contain beta granules, numerous mitochondria and a lamellar type of ergastoplasm.

Hyalin Deposition in Islets. Hyalin deposi-

tion within the islets is one of the pathologic changes observed in some diabetic patients. By light microscopy, hyalin appears as an amorphous protein which also contains lipid and is interposed between the islet cells and their adjacent capillaries [*33*]. Application of electron microscopy to the study of islet cells in diabetic patients is difficult because of the necessity of obtaining pancreatic tissue within a few minutes after its surgical removal from the patient and because of the reluctance of surgeons to obtain biopsy specimens from the pancreas. We have recently examined with electron microscopy hyalinized islets in pancreases obtained at autopsy from diabetic patients. Because of autolysis, preservation of the tissue is relatively poor, however it is possible to observe the hyalin and to determine its relationship to the capillaries and surrounding islet cells. These studies indicate that hyalin is composed of a mass of small fibrils interposed between the two basement membranes separating the capillaries from the islet cells. These fibrils resemble those in beta cell tumors. Occasional collagen fibers are present, but the bulk of the material appears to be composed of small fibrils. The composition and source of these fibrils in the hyalinized islets and their possible role in the pathogenesis of certain types of diabetes in man are unknown at the present time. The fibrillar substance might constitute a potential barrier which could diminish the rate of passage of insulin from the beta cells to the capillaries.

SUMMARY

Electron microscopic criteria have been established for the identification of all the different types of islet cells. A remarkable difference in the ultrastructure of beta granules has been found in different species. Immunochemical evidence has been obtained which indicates that endogenous insulin of neoplastic beta cells may be different from normal human insulin. These electron microscopic and immunochemical findings suggest that there may be differences in the storage form of insulin in different species and in neoplastic beta cells.

Electron microscopic studies indicate that beta granules are formed within ergastoplasmic sacs. Beta granules are secreted by a process of ejection from the cell (emiocytosis).

In studies on experimental diabetes, alloxan has been found to exert its initial effect on the ergastoplasm of beta cells of the rat. Studies on glycogen deposition in beta cells indicate that it first accumulates diffusely throughout the cytoplasm. With sustained hyperglycemia, focal deposition is evident and finally massive accumulations occur which displace the normal intracellular organelles of the beta cells.

Hyalinized islets of diabetic patients have been studied with electron microscopy. Hyalin is composed of a mass of small fibrils which is interposed between the two basement membranes that separate beta cells from capillaries.

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Editorial Introduction to Papers by Poel, Cohen and Meier

THE contributions to this symposium by Yerganian and by Meier and their collaborators direct our attention to the occurrence in nature of persistent hyperglycemic states, one of which may prove to be a truer counterpart of diabetes in man than the set of experimentally induced diabetic syndromes with which we are now familiar. It has proved impossible thus far to reproduce in the animal the specific angiopathy and/or neuropathy, nor has it been possible to dissect the prediabetic state. The study of sporadic spontaneous diabetes in animals may contribute insight into the origin and the histologic picture of retinopathy. The discovery of a diabetic state, genetically transmitted, in the Chinese hamster offers in addition the feasibility of systematic study of "prediabetes," the effects of pregnancy and the presence of associated pathologic states.

The following excerpt from a recent paper by Meier and Yerganian (*Diabetes*, 10: 19, 1961) will serve as an introduction to the further description of lesions associated with diabetes in the hamster, lesions which bear striking similarities to the condition in man.

"Apart from the fact that spontaneous diabetes is unusual in a rodent species, the similarity of the pathological findings to certain human changes and (alloxan-induced) diabetes in experimental animals is of particular interest. The disease is primarily pancreatogenic, the pathological changes consisting of severe damage, vacuolar ("hydropic") degeneration, and deficiency of beta cells; this is borne out by insulin assays. A concurrent pathological finding is diffuse intercapillary glomerulosclerosis associated with deposition of PAS-positive staining material, and leading to uremia in some animals. Polyuria and polydipsia are usually the most prominent clinical signs; some hamsters excrete up to 70 ml. of urine in twenty-four hours.

"Considerable evidence has accumulated to indicate that the disease is hereditary. Diabetes arose spontaneously during the course of inbreeding at a time when many of the sublines of four major families approached the fourth generation of continuous brother-sister mating. The average frequency of genic homozygosity was estimated to be 65 per cent at that time. Attempts at maintaining an extensive breeding program for diabetic hamsters have, so far, been successful. To date, four generations of diabetic hamsters have been obtained with the incidence of diabetes increasing sharply from the fourth to eighth generation, up to 90 per cent of the offspring (e.g., JBY family) becoming diabetic later in life. The degree of penetrance, severity of the disease and age of onset vary greatly among families. Maintenance of fertility of diabetic parents was an ever present obstacle. Also, there were losses of up to 20 per cent of the animals during pregnancy (abortions, resorption) and live-born animals (death soon after delivery, cannibalism). Repeated daily mating tests and treatment considerably improved the number of pregnancies and successful deliveries since the estrus cycles of each female varied greatly. Many animals are totally dependent upon careful control of diabetes for continued existence and maintenance of fertility."

R. L.

Adenocarcinoma of the Pancreas in Diabetes-Prone Chinese Hamsters*

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THE Chinese hamster, Cricetulus griseus, was introduced in the laboratory as an animal for cytogenetic studies because of its comparatively low chromosome number [*1–3*]. Its value as an experimental animal was broadened markedly when spontaneous diabetes mellitus, an unusually rare disease in rodents, was reported to occur with high frequency (65 to 90 per cent) in some inbred strains [*4–6*]. The finding of diabetes mellitus may be related to this brief communication, on the occurrence of adenocarcinoma of the pancreas in the Chinese hamster. Like diabetes, spontaneous adenocarcinoma of the pancreas has been encountered rarely among other laboratory rodents. This malignancy has been reported to have a significant statistical association with diabetes in man [*7*]. The possibility that the association may not be merely one of chance is strengthened by the fact that, despite its rarity in laboratory animals, it is among the first spontaneous tumors to be reported for this species in a partly inbred, diabetes-susceptible strain.

MATERIALS AND METHODS

Three female hamsters of a diabetes-susceptible line, inbred for nine to eleven generations in the Boston colony, received repeated skin applications of 3,4-benzopyrene to determine their susceptibility to induction of skin tumors. Each application consisted of approximately 90 μg. of 3,4-benzopyrene in a 0.0075 ml. drop of toluene, applied to the shaved interscapular skin. Applications were made three times a week for twenty-seven weeks. Past experience with the same technic applied to mice indicated that carcinogenic contamination of areas other than the interscapular skin due to licking, rubbing or scratching by the test animal or its cage mates was insignificant [*8*]. No systemic tumors other than topical ones of the dorsal skin were ever induced in mice with this type of exposure to benzopyrene [*9*].

RESULTS

Small papillomatous growths were observed on the interscapular skin of the three hamsters after twenty, twenty-one and twenty-seven weeks of exposure, respectively. Despite the uniformity of exposure employed, three different types of tumors were induced, i.e., a squamous cell carcinoma in one hamster, a basal cell carcinoma in another and a melanoma of the dorsal skin and subcutaneous tissues which metastasized to a regional lymph node in the third. Incidental findings included an adenomatous pancreatic nodule in the hamster with the basal cell carcinoma, and a well differentiated adenocarcinoma of the pancreas in the animal with the melanoma. (Fig. 1, 2 and 3.) The pancreatic



FIG. 1. Photomicrograph of a section showing primary pancreatic cystadenocarcinoma in a female Chinese hamster compressing the pancreatic duct and normal glandular tissues.

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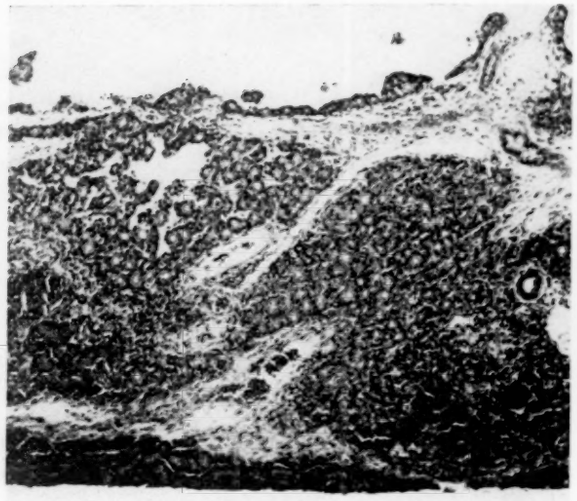


FIG. 2. Photomicrograph of the same tumor: marginal area of a pancreatic tumor cyst, showing bud-like proliferations into the cystic space, invasion and compression of normal pancreatic tissue, and duct-forming components of this carcinoma.

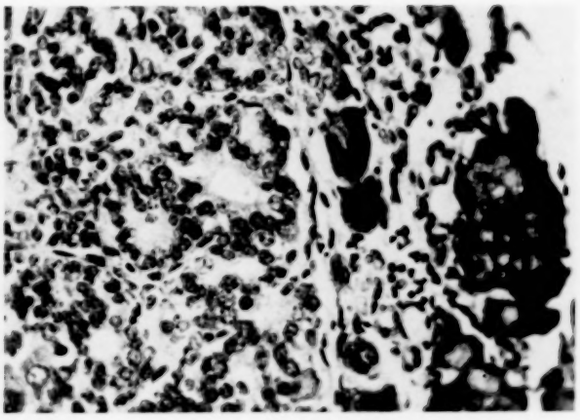


FIG. 3. Photomicrograph of tumor at higher magnification showing duct-forming components and remnants of normal pancreatic acini.

carcinoma consisted of two neoplastic components, one of which suggests a ductal and the other an islet cell origin.

COMMENTS

The incidental finding of pancreatic neoplasms among these hamsters is unusual in at least two regards: (1) the tumors were observed in a very small sampling of animals, and (2) they occurred in three year old females of a partly inbred strain in which a high incidence of diabetes mellitus developed after the fourth generation of inbreeding. The diabetic animals of this strain usually survive for a period of one year or less, even with supportive therapy.

The rarity of spontaneous pancreatic carcinomas in laboratory rodents was first reported by Slye and associates [*10,11*] who found only two primary pancreatic tumors in 125,000 mice autopsied. Both tumors were presumed to be of islet cell origin. One islet cell adenoma in a mouse was also reported by Hueper [*12*]. A recent review of spontaneous tumors in guinea pigs makes no reference to neoplasms of the pancreas in that species [*13*], while a survey of transplantable and transmissible animal tumors by Dunham and Stewart [*14*] lists only an undifferentiated carcinoma of the pancreas which arose spontaneously in a rat. Among Syrian hamsters, a species that is phylogenetically different and not to be confused with the Chinese hamster, an adenocarcinoma of the pancreas was found by Fortner [*15*] in one female among three hundred animals examined. Pancreatic tumors have been induced in mice and rats by several investigators [*16–19*] but in these instances the resultant tumors have been described as predominantly pancreatic adenomas, squamous cell carcinomas or sarcomas. The exposure of the hamsters reported in this communication was such as to suggest a "spontaneous" (i.e., unknown) etiology, rather than induction with benzopyrene.

A colony of hamsters was started in England (Harwell colony) with a nucleus derived from the diabetes-susceptible line of Boston after the fourth generation of inbreeding. Diabetes mellitus has not yet been observed in the Harwell colony, although we have been advised that pancreatic tumors have been seen [*20*]. The association of diabetes with pancreatic tumors through a common inbred stem-line suggests a parallel to the statistical association of the two diseases in man. Cancer of the pancreas was reported to be six to ten times more common among diabetic persons than in the general population [*7,21*]. In a survey of 256 patients with carcinoma and diabetes, 13 per cent were shown to have pancreatic carcinomas, a frequency six times that for the population at large [*21*]. Further work in the laboratory may help to clarify the nature of this association, and the environmental and familial inheritance patterns which may predispose towards one or the other disease. However, such investigations must await the production of more of these interesting experimental rodents than are currently available for long-term studies.

SUMMARY

An adenocarcinoma of the pancreas and a pancreatic adenoma were observed in two of three three year old female Chinese hamsters obtained from a diabetes-susceptible strain that was inbred for nine to eleven generations. An unknown etiology is suggested for the tumors, even though the three animals had received repeated applications of 3,4-benzopyrene to the interscapular skin. The unusual occurrence of these heretofore rare tumors of laboratory rodents, in a diabetic line of Chinese hamster, supports previous indications of a statistical association between diabetes and pancreatic adenocarcinomas. The nature of the association is yet to be clarified.

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Periodontal Pathology in a Strain of Chinese Hamster, Cricetulus griseus, with Hereditary Diabetes Mellitus*

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The relationship between diabetes mellitus and severe periodontal disease has been recognized clinically for many years [*1–12*]. Patients with diabetes mellitus appear to be highly susceptible to the development of inflammatory disease of the gingival tissues, which progresses rapidly to involvement of the underlying supporting tissues of the teeth. The progressive lesion involves extension of the gingival inflammation and regressive changes in periodontal membrane and alveolar bone, together with periodontal breakdown. There is a downward extension of the epithelial attachment uniting gingiva to the tooth, so that the normal gingival crevice becomes deepened to form a periodontal pocket. Clinically, the condition is referred to as periodontitis, or pyorrhea, and is characterized by varying amounts of gingival recession, periodontal pockets with purulent exudate, gradual loosening of the teeth and eventual tooth loss. While periodontal disease is commonly seen in middle-aged and older groups, it is frequently a feature of childhood and adolescence in diabetic persons. Although it can be successfully treated by various surgical and curettage procedures, it has been recognized for many years that even so-called "controlled" diabetics persons have poor tissue healing, so that periodontal therapy is often far from successful.

The relationship between diabetes mellitus and periodontal breakdown is not well understood at this time. The diabetic state apparently lowers the resistance of the supporting tissues of the tooth so that localized gingivitis evolves to a periodontitis. Gingivitis is the result of local irritants in the mouth, usually calculus. Since 1902 [*20*] it has been recognized that generalized pathologic processes produce osteoporosis of alveolar bone which becomes a favorable environment for the development of extensive periodontal breakdown, consisting of interaction of local and systemic factors which may be used to explain severe periodontal reactions in diabetic patients [*13*].

For many years, information on periodontal disease in diabetic patients was related entirely to clinical observation. Experimental work was carried out but the results did not contribute to the general understanding of the problem. Observations on the periodontium of alloxan-treated rats revealed a tendency toward varying degrees of osteoporosis of alveolar bone, but no alteration was found in the nature and incidence of gingival inflammation [*14*]. An entirely new research approach was made possible by Meier and Yerganian [*15–18*], with the development of a strain of Chinese hamsters with spontaneous hereditary diabetes mellitus. As a result of histopathologic study of the periodontal tissues by Cohen, Shklar and Yerganian [*19,20*], it was found that severe periodontal disease developed in these animals.

The Chinese hamster is a particularly appropriate animal for dental research. The three molar teeth are in excellent alignment for histologic sectioning. Both periodontal and pulpal tissues are susceptible to inflammatory

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changes, while the alveolar bone and connective tissue appear to be extremely sensitive to systemic disturbances.

METHODS

Twenty Chinese hamsters (Cricetulus griseus) with spontaneous hereditary diabetes served as the basis for this study. The duration of the diabetic state ranged from twenty-nine days to fourteen months. All diabetic animals received insulin replacement therapy. Fifteen normal animals were utilized as controls.

The teeth of both diabetic and normal animals were viewed with a dissecting microscope. The jaws of both diabetic and normal animals were decalcified by electrolytic technics and sectioned in paraffin.

The present examples of spontaneous hereditary diabetes mellitus were derived primarily from representatives of the BUY family of inbred Chinese or striped-back hamsters (Cricetulus griseus) maintained by one of us (G. Y.). In the animals discussed herein diabetes developed during the eighth to fourteenth generations of brother-sister matings. Previously, other partly inbred strains, notably the VSY, JFY and JBY families, first revealed the diabetic syndrome at approximately the fourth generation of inbreeding. The juvenile form appeared with increasing frequency until the eighth generation among the JBY animals, only to be eliminated because of the outright loss of the genetic pool when the intensive chronic symptoms prevented siblings from reaching maturity.

PERIODONTAL FINDINGS

Normal Animals. In the normal animals the three molars were seen to be in alignment. (Fig. 1A.) The gingival tissue in the interdental areas was attached to the teeth at or close to the cemento-enamel junction. The gingival tissue presented a knife-edge between the molar teeth. The epithelial attachment was broad and the sulcus depth appeared minimal. The molar teeth were characterized by a distal drift. The septums presented appositional reactions on the distal and resorption on the mesial aspects. The periodontal membrane was highly cellular and contained numerous small vascular channels. The gingival tissue normally presented some chronic inflammatory infiltration beneath the epithelium.

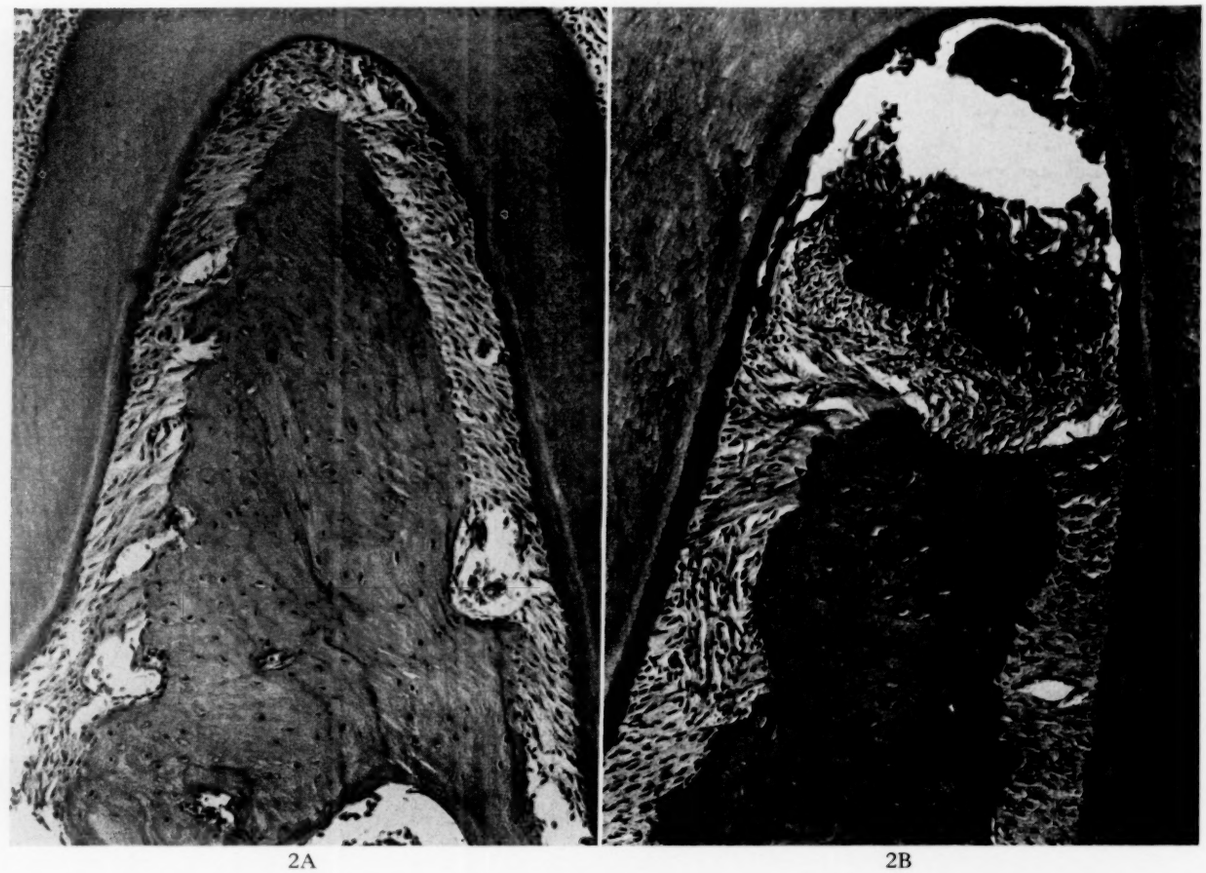
Diabetic Animals. The striking alterations in the diabetic animals are calculus-like deposits on the teeth, migration of the epithelial attachment with splitting of the attachment and pocket formation, alveolar resorption and extensive inflammatory infiltration. All the diabetic animals present some change from the normal. The degree of variation from normal ranged from mild gingival inflammation and epithelial hyperplasia to severe periodontal pocket formation with alveolar resorption. The periodontal alterations are seen in two areas, the interradicular area and the interdental area.



FIG. 1. A, low power photomicrograph of molar region of normal Chinese hamster. Note the alignment of the three molar teeth, and the regular appearance of the pulpal tissue and the gingiva in the interdental areas. Note also the contour of the bone between the roots of the first molar tooth (left). B, low power photomicrograph of the molar area in a diabetic animal. A pulpal abscess is present in the second molar tooth. Note the bifurcation involvement with epithelial migration and calculus deposition in the inter-radicular area of the first molar tooth (left). Hematoxylin and eosin stain.

Interradicular area: The interradicular area of the mandibular first molar teeth was selected as an area for study. Eruption phenomena are usually minimal in relation to the first molar.

Normally, the interradicular septum of bone and the periodontal membrane fill this space. (Fig. 2A.) In diabetic animals the gingival tissue has undergone recession so that the epithelium was not attached to the cementum of the roots. The extent of the bifurcation involvement varied. In several animals the bifurcation was open and



2A 2B

FIG. 2. A, medium power photomicrograph of interradicular bony septum of first molar tooth in normal animal. Note the regular morphology of the periodontal membrane and the crest of the interradicular alveolar septum of bone. B, medium power photomicrograph of interradicular area of first molar tooth in diabetic animal. There is epithelial migration and periodontal pocket formation as the epithelial attachment moves apically. A chronic inflammatory infiltration is present beneath the epithelium and there is resorption of the alveolar crest of bone. Note the calculus deposition. Hematoxylin and eosin stain.

a calculus-like deposit was noted on the cementum. (Fig. 2B.) This deposit was composed of filamentous bacteria and appeared to have been calcified to some extent. The epithelial attachment presented apical migration along the cementum. There appeared to be a split in the epithelial attachment and the formation of a periodontal pocket in this manner. The connective tissue was infiltrated with chronic inflammatory cells and the collagen fibers of the periodontal membrane were distorted, irregular and reduced in number. The alveolar crest was flattened or concave, indicating resorption. Osteoblastic activity was noted on the distal portion of the alveolar septum in both normal and diabetic animals.

Interdental area: In the interdental areas, the reaction was usually more severe. The interdental septum between the first and second molar teeth was selected for study. The epithelial attachment of the gingiva presented apical migration along cementum, splitting and periodontal pocket formation, usually on the mesial. (Fig. 1B.) The periodontal membrane presented a reduction in collagen fibers and a reduced cellularity. The alveolar margin stained deeply and a notable reduction in osteoblastic activity was observed on the distal portion. In most of the diabetic animals there was an obvious reduction in alveolar height. Deposits on the teeth were again in evidence.

COMMENTS

The severe periodontal involvement in these diabetic animals corresponds closely to our experience in man with diabetes mellitus, in whom severe periodontitis is noted, particularly in children and young adults. Delayed healing also

is observed in cases of periodontitis in diabetic patients who have been treated with either surgery or curettage.

Observations are now under way to relate the severity of the periodontal involvement in the Chinese hamster to the severity of the diabetic state.

From our observations, several interesting findings emerged in relation to the development of periodontal disease. The periodontal pockets appeared to develop by epithelial proliferation and a split in the epithelial attachment with eventual degeneration of the cells on the cemental surface. The features of the periodontal membrane and alveolar bone suggest that diabetes acts upon the periodontal tissues in an antianabolic manner, similar to many other systemic alterations studied in relation to the periodontium of experimental animals. The severe bone loss in the diabetic hamster is presumably the result of local irritation and pocket formation acting on bone and connective tissue altered by the diabetic state. Our findings appear to substantiate Znamensky's [*13*] concept of the interaction of local and systemic factors in periodontal disease related to systemic disturbances or disease states.

SUMMARY

Severe periodontal disease was noted in a strain of Chinese hamsters with hereditary diabetes mellitus. The periodontal involvement was characterized by pocket formation, inflammation and alveolar bone resorption.

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Comparative Aspects of Spontaneous Diabetes Mellitus in Animals*

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HAVING recently reviewed the subject of spontaneous diabetes mellitus in animals, repetition of some of the observations made is unavoidable; however, in the present text certain additional aspects, not previously covered, will be discussed and greater emphasis placed on comparative microscopic and clinicopathologic findings. For the sake of completeness, all references to reports on spontaneous diabetes mellitus in domestic animals (grouped according to animal species) have been added to the bibliography. Only those publications directly quoted from and general reference matter have been incorporated in this presentation. The very extensive literature concerning surgical and drug-induced diabetes has not been evaluated.

INCIDENCE

Estimates of the relative frequency of spontaneous diabetes in larger domesticated animals vary considerably, although the reported incidence for dogs, for example, ranges from 1:260 to 1:800, and that for cats, approximately 1:1,000 to 1:1,500. Personal observations of some 30,000 dogs and cats would indicate that the disease is much more frequent, roughly 1:200 for dogs and 1:800 for cats. The relative frequency of diabetes in cattle, horses, pigs and sheep is unknown, but judging from the literature the condition seems to be infrequent. Although the entire veterinary literature contains probably fewer than one hundred references to spontaneous diabetes mellitus, and most of them describe only single cases or a few cases each, it is not to be inferred that this condition is rare.

Perhaps of greatest importance with respect to the utilization of spontaneously diabetic animals in diabetes research is the observation of a hereditary form in Chinese hamsters. Continued brother-sister inbreeding has increased the genic homozygosity from 65 per cent at the time of discovery to nearly 100 per cent. Characteristically for each line the degree of penetrance, severity and onset of the disease varies; one line being comparable (if such analogy is permissible) to juvenile diabetes since the islet disorder starts as early as fourteen days after birth. There is thus at hand an experimental animal in which genetic studies can readily be made; moreover, among offspring there is a comparable degree of measurable biochemical abnormality (hyperglycemia, ketosis and the like).

CLINICOPATHOLOGIC ASPECTS

Diabetes mellitus in animals may be due to either primary (idiopathic) or secondary islet disease; whether primary or secondary would be difficult to assess clinically, however one or the other type may be suspected from previous experience. In a *horse* the clinical course and histopathologic lesions observed in the pancreas and pancreatic islets were interpreted as a sequence of influenza, a viral disease of horses. Most of the cases of diabetes mellitus in *cattle* followed chronic infections, notably tuberculosis. Although primary or secondary islet disease appears not to have been observed in *sheep*, hyperglycemia and "diabetic coma" have appeared in fed lot sheep during rapid fattening. Diabetes in *pigs* secondary to pancreatitis and acute enteritis has been reported; viral enteritides are common in pigs.

In the *dog*, the disease occurs in all age groups; however, there is a marked peak in incidence after eight years of age. The majority of diabetic dogs are females, especially neutered females. Diabetes is less frequently a disease of the islet tissue proper than a probable sequel to acute and chronic (relapsing) pancreatitis which is

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common in obese animals. In view of the fact that the canine pancreas usually has two excretory ducts, the main duct entering into the duodenum about 1 cm. posterior to the bile duct, pancreatitis is not thought to result from bile reflux. Rather it has been suggested that since the pancreatic acinar tissue is actively involved in protein synthesis, a relative lack of lipotropic materials in obese animals may cause a metabolic disturbance and collapse of the pancreatic acini. The islets may become secondarily affected, following autodigestion and inflammation. The relationship of obesity to diabetes mellitus will be considered in more detail further on. Some dogs appear to be predisposed to repeated bouts of pancreatitis, diabetes occurring only during recurrent attacks. Symptoms of acute pancreatitis are abdominal pain and peripheral circulatory collapse. If the disorder persists for several days there will be hyperglycemia due to destruction of islet tissue. In chronic pancreatitis, polydipsia and polyuria frequently are first noted, or signs of digestive disturbances consisting of ravenous appetite or anorexia prevail. In a few weeks' time, following apparent recovery, symptoms referable to diabetes mellitus with steatorrhea occur. Although the signs of pancreatic fibrosis are usually similar to those of chronic pancreatitis, the condition is of more gradual onset; the symptomatology is indistinguishable from diabetes with primary islet cell lesions. It should be mentioned that in all cases of pancreatic carcinoma reported in the literature, and from personal observation, diabetes has never been found, in contrast to the situation in man.

Diabetes in *cats* is considerably more common in males (mostly young), than females and is of a secondary type. There is a causative relationship to infection, e.g., bite wounds and alveolar abscesses of teeth, to which young fighting tomcats are considerably more subject than females.

In addition to hyperglycemia, various degrees of lipemia occur in all animals except in the Chinese hamster; acetonemia is present also in all animals, but is very scarce in the Chinese hamster. Most clinical laboratory experience relates to dogs; technics for assay of pancreatic (exocrine) function and glucose tolerance tests are almost routine in many university veterinary clinics. The relevant procedures have been published [*1*]; exocrine function tests consist of vitamin A uptake, viokase or a combination of both, and amylase determinations. Typical glucose tolerance curves for a normal dog and one suffering from diabetes are illustrated in Figure 1.

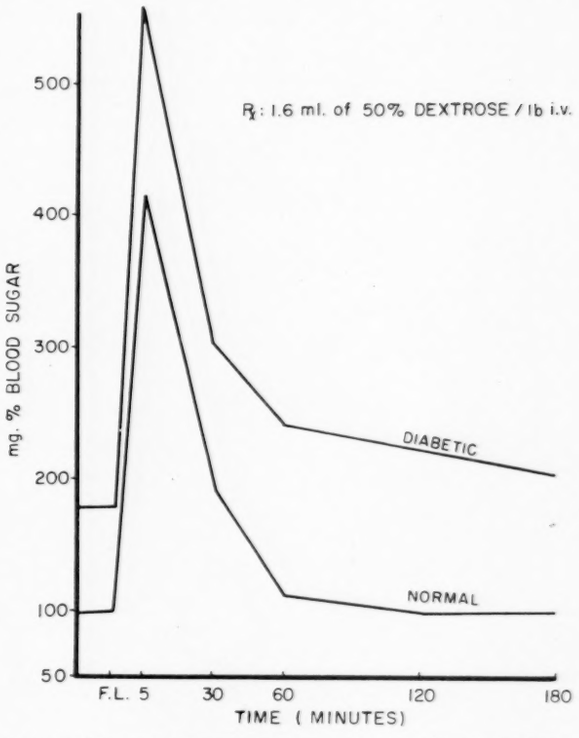


FIG. 1. Glucose tolerance curves of normal and diabetic dogs.

Polyuria and polydipsia are usually the most prominent clinical signs of spontaneous hereditary diabetes mellitus in *Chinese hamsters*. Of particular interest is the fact that electrophoretic patterns of serum proteins in families with a high incidence of spontaneous diabetes reveal alpha$_2$ globulin levels to be two to three times the normal values. Since the alpha$_2$ serum proteins are increased even prior to the onset of clinical diabetes and microscopic evidence of intercapillary glomerulosclerosis, this finding may be used as a genetic marker in breeding experiments designed to increase the incidence of spontaneous diabetes.

HISTOPATHOLOGIC FINDINGS

Among the primary islet changes, "*hydropic*" *degeneration* and *hyalinization* prevail in Chinese hamsters, dogs and also some cats of both sexes. Islet changes consequent to secondary *amyloidosis* are most frequent in cattle and young male cats, and acute and chronic *pancreatitis* commonly occurs in obese dogs. It has also been demon-

TABLE I
ISLET SIZES

Animal	No. of Islets Measured	Islet Sizes (μ)						
		−50 %	51–100 %	101–150 %	151–200 %	201–250 %	251–300 %	301– %
Dog.	2,600	18	62.5	12.3	5.7	1.0	0.3	0.15
Cat..	500	12.6	55.0	20.4	9.4	1.6	0.4	0.6

TABLE II
DISTRIBUTION COUNTS (ISLETS/CU. MM.)

Animal*	Splenic Portion	Middle Portion	Duodenal Portion	Total Average
Dog.....	67–223 (164)	82–222 (134)	37–172 (88.6)	130
Cat.....	111–159 (138.4)	86–149 (116)	72–120 (87)	113.6

NOTE: Figures in parentheses represent the average.
* Twenty-six dogs and cats.

strated that in dogs with idiopathic diabetes, as in human subjects with diabetes, the argentophilic alpha cells of the islets increase in number. The proportion of alpha to beta cells, which normally is about 1:4, may become 3:4.5.

Among the various microscopic changes described for *cats*, other than those mentioned, are *diminution* in number and/or size of islets, fibrosis and neoplasia(?) of acinar tissues. In *dogs* also, diminution in the size and number of islets and even almost complete absence of islet tissue have been held responsible for diabetes. This evidence was based on distribution counts, islet measurements, percentage of pancreas comprised by islets and proportion of islet tissue to exocrine acinar tissue. Some of the normal histoanatomic observations which formed the basis for comparison (with diabetic dogs and cats) are presented in Tables I, II and III. It must be remembered, however, that in the dog there are normally fewer islets in older animals than in young ones, indicating decreased ability of regeneration with aging.

The main cause of diabetes in the Chinese hamster is a disorder of beta cells, characterized by degranulation, vacuolar (hydropic) degeneration and beta cell deficiency. The similarity of the pathologic findings to certain changes in man and especially in experimental animals with alloxan-induced diabetes seems of particular interest.

TABLE III
PERCENTAGE OF PANCREAS COMPRISING ISLETS AND PROPORTION OF ENDOCRINE TO EXOCRINE TISSUE

Portion	Percentage		Proportion	
	Dog	Cat	Dog	Cat
Splenic.....	1.65	1.67	1:65.5	1:71
Duodenal...	0.52	0.63	1:240	1:185

Although diabetic *glomerulosclerosis* occurs in animals, the typical hyaline lesions of nodular intercapillary hyalinization are not observed. The lesions appear to involve a more diffuse thickening of the capillary walls, rather than localized hyaline deposits. However in the dog, glomerulosclerosis perhaps develops as a further progression of renal arteriolar sclerosis, which is quite frequently observed, associated with diabetes. Although with age there are some morphologic alterations in the glomeruli of Chinese hamsters, they differ strikingly from those observed to be associated with spontaneous hereditary diabetes mellitus, both qualitatively and quantitatively. In diabetic intercapillary sclerosis there is significant thickening or splitting of the basement membranes and intercapillary deposition of periodic acid-Schiff-positive hyaline masses. The extent of the lesions correlates both with time of onset and severity of diabetes, e.g., in one line of hamsters with diabetes characteristically manifest at day eighteen of life, intercapillary glomerulosclerosis developed in progression with the diabetic condition as judged by histologic examination of the pancreatic islets and glucosuria. The most severe degree of glomerular change may be seen at one month of age, many animals dying as a result of uremia. The point has been made that perhaps the renal lesion is primary and not necessarily consequential to degeneration of the beta cells. In this connection the observation of increased $alpha_2$ globulin levels, two and three times the normal values, in serum electrophoretic patterns of families with a high incidence of spontaneous diabetes is important. Upon random hybridization of such families by single or double crosses normal levels of $alpha_2$ globulins are re-established.

The problem of *retinopathy* in diabetic animals has not been studied sufficiently; however,

occular changes have been observed in dogs and are currently being investigated in the Chinese hamster.

OBESITY AND HYPERGLYCEMIC SYNDROMES

There are no reports of spontaneous diabetes mellitus in mice and rodents in general, except for spontaneous hereditary diabetes in Chinese hamsters. However, hyperglycemic syndromes have been described in certain stocks of mice. One, a mutation (ob), occurred in the V stock of the Roscoe B. Jackson Memorial Laboratory causing extreme obesity and, in the homozygote, sterility [*2*]; some obese males could breed when kept on a restricted diet and otherwise sterile females could be made fertile by the administration of gonadotropic hormones and induction of ovulation [*3,4*]. The other type is a "yellow" mouse such as mice of strain YBR/Wi; mice possessing the Y′ gene (other than being yellow coated) become obese when fed enriched diets [*5*].

Aside from a variety of pathologic changes occurring especially in obese and non-obese mice of YBR/Wi strain, common to both the Obob and YBR/Wi mice are conspicuous hyperplasia and hypertrophy of the pancreatic islets. Differential staining (e.g., post-coupled benzilidine reaction, Gomori's aldehyde fuchsin and chrome alum hematoxylin-phloxin) has failed to reveal deviations from the normal pattern (obese mice); similarly, no alterations in alpha: beta cell ratios and cytoplasmic granulations are detected in the YBR/Wi mice.

Both of these types of hyperglycemia are resistant to treatment with insulin. Details of the biochemical aberrations in hyperglycemia obese mice have been summarized; they are similar to the metabolic abnormalities in chemically-induced (goldthioglucose) obese mice. Hepatic glucose metabolism has been studied in obese hyperglycemic mice. The findings were as follows: oxidation of glucose (glucose-1-C^{14} and glucose-6-C^{14}) to carbon dioxide was normal (30 per cent of normal in diabetic Chinese hamsters), glucose incorporation into glycogen was only about one-fifth of normal (9 per cent of normal in the hamster) while incorporation into fatty acids was over 200 per cent of normal (versus 15 per cent in the hamster).

It seems most likely that hyperglycemia in genetic obesity is similar to obese diabetes in man. Also the diabetes-like condition in feed lot sheep may be analogous.

A recent study suggests a definite interrelationship of diabetes mellitus and obesity in dogs, as in man [*6*]. In a statistical evaluation of the necropsy findings in 10,993 dogs, 971 were obese and 167 had diabetes mellitus; of the diabetic dogs 100 were obese. Obesity tended to occur at a slightly earlier age than diabetes, and significantly greater numbers of females than males were affected. Analysis of the breed distribution relative to the general dog population revealed that certain breeds were significantly predisposed or resistant to obesity and diabetes. Particularly noteworthy among these were two acromegalic breeds (Rottweiler and St. Bernard) since it had been previously demonstrated that in acromegalic dogs the pituitary contains larger numbers of acidophils than in non-acromegalic dogs [*7*]. Although these cells are considered to be the source of pituitary diabetogenic hormone, the possibility of a pituitary effect must be viewed with caution since certain non-acromegalic breeds are also highly predisposed to obesity and diabetes whereas acromegalic Great Danes are not. However, a hereditary susceptibility may still play a role in certain breeds; such a condition has been reported for a family of Shetland Sheepdogs, in which hereditary obesity appeared as a recessive Mendelian character [*8*].

CONCLUDING REMARKS

Comparative pathologic studies on spontaneous diabetes mellitus in domestic animals warrant greater attention. More information regarding etiology, pathogenesis and the like is required, with objective statistical analysis (incidence, species and breed distribution). New facts about the general problem of diabetes may be learned from this approach.

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Plasma Insulin in Health and Disease*

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TRADITIONALLY, hormone concentrations in plasma have been estimated by comparing the biologic effects produced by plasma and by a standardized hormone preparation in some sensitive system specifically influenced by the hormone. More recently, immunologic approaches have been exploited with increasing application. In the immunoassay technic, the effect of plasma is compared with that of a standardized hormone preparation in the reaction with antibodies directed specifically against the hormone.

Ideally, a bioassay is to be preferred since hormonally active and immunologically reactive sites of the hormone molecule may not correspond and it is the biologic potency that is of interest. Thus a hormone molecule, rendered biologically inactive by some metabolic or degradative change, may yet retain some or all of its immunologic integrity. Although the possibility of interference by immunologically reactive but biologically inert products of hormone metabolism cannot therefore be disregarded,† immunologic specificity is likely to exceed greatly biologic specificity. Furthermore, some of the newer immunologic technics are capable of detecting hormones with far greater sensitivity and precision than have yet been demonstrated with most bioassay methods.

Used for a longer time and more widely in clinical practice than any of the other peptide hormones, insulin has attracted great interest from physiologists and biochemists alike and has on this account enjoyed many advantages over the other hormones. First crystallized about thirty-five years ago [*1*], shortly after its isolation by Banting and Best [*2*], insulin was the first protein to succumb to a complete analysis of amino acid sequence by Sanger and his colleagues [*3*]. For years, insulin has been available as one of the purest of proteins and its chemical properties and physiologic effects have been studied in great detail. Yet its physical state in the blood, at least with respect to molecular size, remains uncertain, and its mechanism of action on cells is still open to speculation [*4*], although some lines of approach [*5,6*] in attacking the latter problem are encouraging. Even the question as to whether insulin has any direct effect on the liver is a subject of current dispute. It is perhaps therefore not surprising that, in spite of the labor of many investigators over the past twenty years, the assay of plasma insulin is still a subject of active interest and one in which many lacunae remain to be filled.

The earliest attempts to measure plasma insulin involved the administration of plasma to small animals sensitized to insulin by removal or destruction of one or more of the endocrine organs [*7–10*]. These methods may be classified under the heading, "*in vivo* bioassay." Groen and his associates [*11*] were the first to use an *in vitro* system to measure plasma insulin. These workers showed that plasma from normal subjects mimicked the effect of insulin in increasing glucose uptake by the rat diaphragm, that plasma from depancreatized dogs had no such effect, and that sulfhydryl agents known to inhibit activity of crystalline insulin [*12*] also abolished plasma insulin-like activity. Furthermore, Randle and Taylor [*13*] have shown that insulin antiserums block the effect of plasma insulin-like activity on the diaphragm. The diaphragm technic has been employed extensively by Groen, Randle, Vallance-Owen, and their collaborators and by others [*14–23*].

† For example, it has been demonstrated that desoctapeptide insulin, an hormonally inactive product of enzymatic hydrolysis of insulin in the test tube, is immunologically reactive with insulin antibodies (see companion paper, page 882). However, whether similar peptides, even if produced by metabolic processes *in vivo*, gain entry into the circulation remains a matter of conjecture.

* From the Radioisotope Service, Veterans Administration Hospital, Bronx, New York. Manuscript received September 9, 1961.

More recently, Martin, Renold and Dagenais [*24*] introduced another *in vitro* system, the epididymal fat pad of the rat, for the assay of plasma insulin, demonstrating that glucose uptake by this tissue is extraordinarily sensitive to the influence of insulin. Finally, several immunologic approaches to the assay of insulin have been reported. Arquilla and Stavitsky [*25*] found that the degree of hemolysis of insulin-sensitized erythrocytes in the presence of insulin antibodies could be quantified as an inverse function of insulin concentration pre-incubated with antiserum, and Loveless [*26*] has devised a quantitative assay using skin-sensitivity technics. However, these methods have not been developed with sufficient sensitivity to be useful for assay of insulin in plasma. The reaction between I^{131}-labeled insulin and insulin antibodies, shown to be competitively inhibited in the presence of unlabeled insulin [*27*], also has been applied to the assay of plasma insulin [*4,28–37*]. Improved counting methods and the use of very sensitive antiserums now permit detection of less than 0.1 microunit (μU.) endogenous plasma insulin in man [*35–37*] making this immunoassay by far the most sensitive of all reported insulin assays.

A detailed critical evaluation of the various assay technics, their specificities, sensitivities, advantages and disadvantages, will not be presented here. Some of the problems have been considered in a recent review [*38*] and detailed discussions of individual methods are given elsewhere [*39*]. However, it is important to emphasize that values obtained for plasma insulin by different methods are frequently not directly comparable. In some cases values obtained by different workers employing essentially the same method differ by an order of magnitude [*cf. 38*]. At least part of the discrepancy has been explained by the observation that serum dilution results in an apparent increase in plasma insulin concentration in both the diaphragm [*20*] and fat pad [*40*] methods. Since different serum dilutions have been employed in different laboratories, it is possible to evaluate results in certain clinical conditions only when comparisons are presented from the same laboratory. In the present review it is proposed to present reported values for plasma insulin concentrations in various physiologic and pathologic states, citing the method of assay employed and reference values from the same laboratory when pertinent.

PLASMA INSULIN IN NORMAL SUBJECTS: FASTING LEVELS AND RESPONSE TO GLUCOSE

In an early report, Gellhorn et al. [*8*] estimated an insulin concentration of 200 μU./ml. in whole blood, two and a half hours after feeding, from the fall in blood sugar induced in adrenalectomized hypophysectomized rats. Bornstein and Lawrence [*10*] reported average levels of 340 μU./ml. in plasma two hours after the administration of glucose, using similar animals rendered additionally sensitive by the induction of alloxan-diabetes. Eight years later, Baird and Bornstein [*41*] using plasma, extracted with a mixture of organic solvents, in adrenalectomized, alloxan-diabetic mice, reported values of 800 to 1,150 μU./ml. in normal fasting subjects and 1,700 to 3,700 μU./ml. after feeding. Anderson et al. [*9*] extracted large amounts of plasma from fasting dogs and found only 65 μU./ml. by assay in adrenalectomized, hypophysectomized alloxan-diabetic rats.

As noted earlier, plasma insulin concentrations determined by the diaphragm technic have varied widely. Vallance-Owen et al. [*14,15*] and Seltzer and Smith [*23*] have used undiluted plasma and report average fasting plasma concentrations of about 60 μU./ml. (more recently Seltzer [*42*] has found an average value of 37 μU./ml.); after glucose feeding, concentrations rose five- to tenfold. Assaying plasma at various dilutions, Willebrands et al. [*20*] found fasting levels as high as 4,600 μU./ml. in some cases and Randle [*16*] has reported values of 9,000 to 22,000 μU./ml. two and a half hours after the administration of glucose. Groen et al. [*43*] have attributed the lower values observed with undiluted plasma to the presence of insulin inhibitors, notably adrenalin, the effects of which are lost on plasma dilution. Using mouse diaphragm and plasma diluted 1:3, Oyama et al. [*22*] found plasma insulin concentrations of only 24 μU./ml. in non-fasted subjects, preparing their standards in serum to control the observed effect of serum insulin inhibitors [*44*]. Yet others [*15,21*] have reported quantitative recovery of insulin added to normal serum and thus imply a nugatory effect of inhibitors in normal serum.

The fat pad assay yields values ranging from 33 to 940 μU./ml. for "insulin-like activity" in normal fasting plasma and levels two to four and a half times as high after the administration of glucose, according to the data of Sheps et

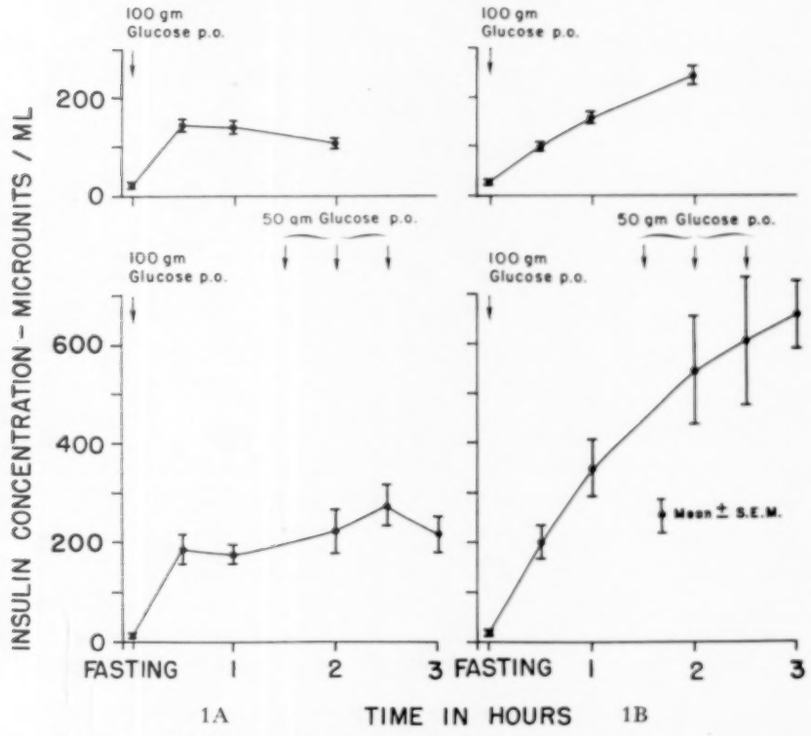


FIG. 1. A, non-diabetic control subjects. B, patients with early maturity onset diabetes. *Top*, mean plasma insulin concentrations before and during a standard 100 gm. oral glucose tolerance test in subjects never treated with insulin (thirty non-diabetic subjects and thirty-eight diabetic subjects). *Bottom*, same as above except that additional loads of 50 gm. glucose were administered during the test at one and a half, two and two and a half hours (four diabetic subjects and five non-diabetic subjects). (From: YALOW, R. S. and BERSON, S. A. *Diabetes*, 10: 339, 1961.)

al. [*45*]. However, Leonards [*46*], Steinke et al. [*47*] and Goldberg and Egdahl [*48*] find that fasting levels of insulin-like activity persist unchanged after pancreatectomy. Of further interest is the observation of Goldberg and Egdahl [*48*] that plasma insulin-like activity of depancreatized dogs is increased after glucose administration. Inasmuch as these dogs were severely diabetic and insulin could not be detected in plasma by immunoassay before or after glucose, it must be concluded that plasma substances with insulin-like activity on the fat pad but without significant hormonal activity *in vivo*, and lacking the ability to react with insulin antibodies, are produced by extrapancreatic tissue. Leonards [*46*] had earlier reported that insulin-neutralizing antiserum had little or no effect on insulin-like activity of normal human serum and the report of Sheps et al. [*45*] indicates that some plasma insulin-like activity on the fat pad persists after treatment of plasma with reduced glutathione. There appears to be little doubt that the fat pad is very sensitive to the action of plasma insulin, but the precise contribution of other plasma factors to the effects observed remains in question.

A relatively large body of data on plasma insulin concentrations in normal man is available from studies with the immunoassay technic [*33,35,36*]. Results in thirty control subjects in the fasting state and following a standard glucose load are summarized in Figure 1. Plasma insulin concentrations averaged 21 μU./ml. (range 0 to 70 μU./ml.) in the fasting state and rose to a mean value of about 140 μU./ml., thirty to sixty minutes after the oral administration of 100 gm. glucose, falling thereafter [*33*]. In non-diabetic subjects subjected to heavier glucose loading or achieving high blood glucose levels because of rapid absorption (e.g., due to gastroenterostomy), considerably higher values were frequently encountered [*33*]. (Fig. 1.) Grodsky and Forsham [*34*] employed a modification of the immunoassay method, using a salt fractionation technic for separation of antibody-bound and free insulin, and were unable to detect insulin

extracted from 5 ml. of plasma in thirty of thirty-two fasting normal subjects, but the lower level of insulin detection by this method is 20 μU./ml. beef insulin; plasma insulin (average concentration 31 μU./ml.) was detected thirty minutes after the intravenous administration of glucose.

DIABETES

Bornstein and Lawrence [*10*], using adrenalectomized-hypophysectomized alloxan-diabetic rats to assay insulin, reported concentrations of 100 to 300 μU./ml. after feeding in diabetic patients not subject to ketosis. These values were almost as high as in normal subjects of the same study (*vide supra*). Plasma insulin was not detected in subjects with juvenile diabetes or in adult diabetic subjects prone to ketosis. Similar findings were reported by Vallance-Owen et al. [*49*] and by Seltzer and Smith [*42*] using the rat diaphragm method. The immunoassay procedure has revealed that early or mild maturity-onset diabetes is frequently associated with high plasma insulin, particularly after the administration of glucose [*32,33*]. Although the insulin-secretory response in diabetic subjects was not as brisk as that observed in non-diabetic subjects, insulin concentrations eventually achieved in this group of diabetic subjects frequently exceeded the levels reached in control subjects, not only during a standard glucose tolerance test but also following a heavier glucose load (Fig. 1), presumably a consequence of the persistence of hyperglycemia. Whether the failure of the blood sugar to respond well to these high plasma insulin concentrations is due to a defective insulin, to the presence of insulin inhibitors, or to "tissue resistance" remains to be determined. In subjects with juvenile diabetes, adult insulin-treated diabetes of greater severity and diabetes associated with long-standing chronic pancreatitis, insulin concentrations measured by immunoassay have been found to be frequently low or absent in the face of marked hyperglycemia [*36*], indicating a virtual absence of mobilizable pancreatic-insulin reserves in such subjects.

OTHER HYPERGLYCEMIC STATES

Higher than normal plasma insulin levels have been reported in acromegaly by both rat diaphragm [*17,50*] and immunoassay [*33,34*] methods and in hyperthyroidism with the latter method [*33*].

HYPOGLYCEMIC CONDITIONS

Idiopathic Hypoglycemia of Childhood. The finding of Cochrane et al. [*51*] that children suffering from hypoglycemia frequently experience a rapid fall in blood sugar abruptly following the oral ingestion of leucine has stimulated considerable research. It has been shown that leucine-induced hypoglycemia is associated with an acute rise in plasma insulin concentration in most cases [*33,52,53*]. Unexplained features of this disorder are the presence of *fasting* hypoglycemia associated with normal plasma insulin concentration and glucose tolerance curves of normal shape but with all values significantly below normal. However, leucine sensitivity of slight degree may be demonstrated in the normal rat [*54*] and can be induced in normal and diabetic human subjects by the prior administration of sulfonylurea drugs [*55*]. Thus, susceptibility to stimulation by leucine appears to be a characteristic of even the normal islet beta cells. It is therefore possible to interpret the response in leucine-sensitive hypoglycemic subjects as an exaggeration of a normal tendency and, further, to suggest that this is accompanied by a similar increase in sensitivity of the beta cell to the level of blood sugar, which may result in release of insulin even when the blood sugar is lower than normal. The further possibility that an abnormal setting of the islet cell "glucostat" exists in other idiopathic hypoglycemic states (Fig. 2) should be subject to investigation by a study of plasma insulin concentrations under appropriate experimental conditions.

Functioning Islet Cell Adenoma. Higher than normal fasting plasma insulin concentrations have been observed in somewhat more than 50 per cent of pancreatic insulinomas, whether measured by rat diaphragm [*56*], fat pad [*57*] or immunoassay (Fig. 2) procedures. Thus, whereas a high fasting plasma insulin concentration, when associated with hypoglycemia may, in itself, be of some diagnostic value, the frequent failure to establish hyperinsulinism in proved cases even on repeated testing [*56*] (Fig. 2) makes it impossible to exclude the diagnosis with certainty by means of plasma insulin determinations.

Schwartz and his co-workers [*58,59*] reported several cases of insulinoma in which acute hypoglycemia was induced by leucine. An accompanying increase in plasma insulin concentration has been demonstrated in such cases [*33,36,60*]. The disappearance of leucine sensitivity follow-

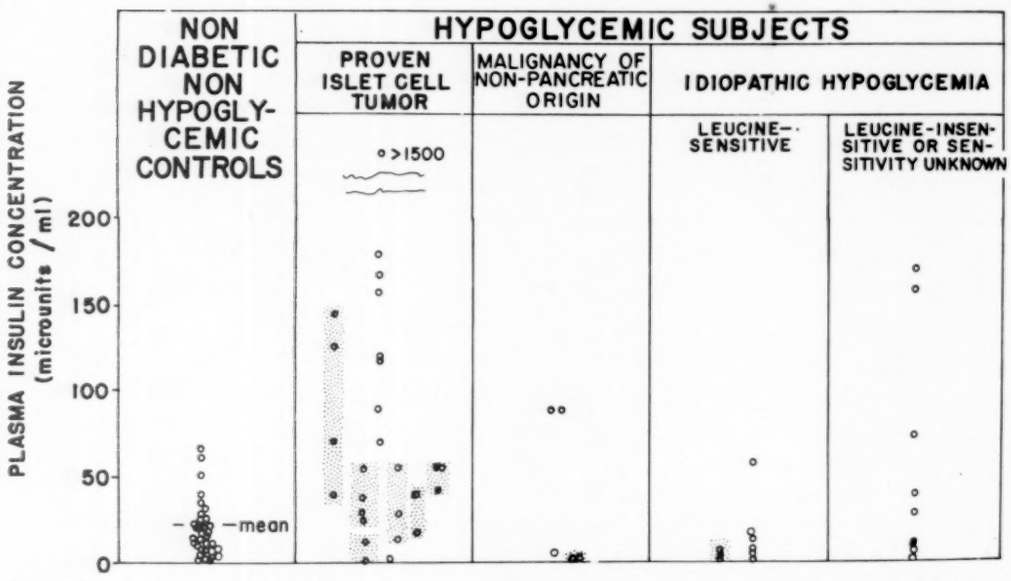


FIG. 2. Insulin concentrations in hypoglycemic subjects are shown only for blood samples in which blood glucose concentration was less than 60 mg./100 ml. All points within a stippled area are from the same subject. From: BERSON, S. A. and YALOW, R. S. In: Detection and Assay of Hormones by Immunochemical Means. Ciba Foundation Colloquia on Endocrinology, Boston 1961, Little, Brown and Co. N.B. In one of the cases shown as hyperinsulinism associated with malignancy of non-pancreatic origin, recent autopsy has revealed a small islet cell adenoma as well as widespread metastatic neuroblastoma.

ing removal of the adenoma [*36,59,60*] indicates that it is the tumor and not the normal islet cell which is being stimulated in these subjects. An abnormal sensitivity of insulinomas to sodium tolbutamide has recently been demonstrated by Fajans et al. [*61*]. Plasma insulin concentrations, determined by immunoassay, were found to increase even more strikingly following the administration of tolbutamide than following glucose administration in some of these patients [*35*], in contrast to observations in normal and diabetic subjects [*62*]. However, these studies have raised more questions than are answered, since the prolonged persistence of hypoglycemia for several hours following tolbutamide administration [*61*], which is not seen in normal or diabetic subjects, is not necessarily associated with persistent hyperinsulinism [*35*].

Other Hypoglycemic Conditions. Fasting hyperinsulinism associated with hypoglycemia has been observed in some cases of malignancy of non-pancreatic origin. (Fig. 2.) Since significant concentrations of insulin in these tumors have not yet been demonstrated unequivocally, in our opinion, the possibility should be considered that these tumors are capable of elaborating some substance capable of stimulating the normal islet beta cell.

The Physicochemical State of Insulin in Plasma. In the past few years there have been many reports purporting to demonstrate the existence of endogenous plasma insulin in some "bound" form, wherein it may be restricted from exhibiting insulin-like activity in certain biologic systems but is free to do so in others. So-called "bound" insulin is occasionally reported to be released by treatment of plasma in various procedures [*63*]. Moreover, it is claimed by some that insulin-I^{131} added to plasma becomes "bound" to a normal serum protein [*64*] whereas others [*63*] report that added exogenous insulin does not behave like endogenous insulin in this respect. Since conflicting evidence has been presented from different laboratories and, in most cases, full details of procedures and results are not presently available, extensive review of the literature on "bound" insulin is neither possible nor warranted at this time. However, in view of the considerable activity and the many reports appearing in this field, a brief discussion is indicated. Investigations in our laboratory have been restricted to studies of insulin-I^{131} in plasma and to assay of endogenous plasma insulin which is immunologically reactive. Without equivocation, it may be stated that results obtained from paper, starch block and

starch gel electrophoresis and from ultracentrifugal analysis of plasma containing insulin-I^{131} have failed to reveal any evidence for binding of insulin-I^{131} to normal plasma proteins, whereas binding of insulin-I^{131} to insulin antibodies is readily demonstrated by any of these technics. Furthermore, after ultracentrifugation for nine hours at 40,000 r.p.m. the supernatant fluid above the sedimentation boundary of serum albumin reveals an *endogenous* plasma insulin concentration (as measured by immunoassay) only about 30 per cent lower than that present in the uncentrifuged plasma, which is in agreement with results obtained on the sedimentation of insulin-I^{131} added to plasma or to buffered solutions containing only albumin or insulin [*36*]. These findings indicate that neither insulin-I^{131} nor *immunologically reactive endogenous* plasma insulin is bound to any normal plasma protein of significant mass. Since similar results were obtained at serum dilutions ranging from 1:2 to 1:50 [*36*], it is unlikely that these observations can be alternatively interpreted in terms of a reversible equilibrium between "bound" and "free" insulin. Furthermore, dilution of plasma over the ranges of 1:2 to 1:20 or 1:10 to 1:100 does not affect the total insulin concentration as determined by immunoassay [*32,33*]. It may be argued, however, that "bound" insulin cannot react with antibodies and should therefore not be measurable by immunoassay. The inability of antiserum to abolish completely the insulin-like activity of plasma on the fat pad [*46,65*] does, of course, indicate that such insulin-like activity is unlikely to be measured by immunoassay, but one might ask what evidence there is that all such insulin-like activity is, in fact, due to insulin itself. The ability of potent antiserums to induce acute severe diabetes in mice [*66*], rats, rabbits and cats [*67*] indicates that at least all *hormonally active* endogenous insulin is reactive with insulin antibodies. Furthermore, in a recent study, Anderson et al. [*68*] have *extracted* insulin from plasma, under conditions which would presumably release "bound" insulin if such existed, and have found by assay, in an *in vivo* system, that the concentration of plasma insulin in the fasting dog is only 30 μU./ml., a value much closer to that obtained with the immunoassay technic than with the fat pad method.

In a recent series of articles Antoniades and his co-workers [*69–72*] have reported that "bound" plasma insulin is capable of exerting an effect on the isolated rat diaphragm only after dissociation of an insulin-protein complex presumed to exist in the plasma. Release of "bound" insulin into the "free" form is said to be effected by incubation with extracts of adipose tissue. Assuming that glucose uptake by the rat diaphragm in the presence of untreated serum represents "free" insulin and that the increase observed in the presence of serum treated with adipose tissue extracts represents "bound" insulin, Antoniades et al. [*72*] presented results indicating that serum from fasting normal and diabetic patients contains insulin principally in the "bound" form, the concentration of which ranges from about 200 to 1,000 μU./ml. in the diabetic patient. Following the intravenous administration of glucose, "total" insulin concentration *decreases* in the majority of diabetic and non-diabetic subjects, the "bound" insulin virtually disappearing from the circulation within ten minutes in most patients while the concentration of "free" insulin rises concomitantly in all subjects [*72*]. Antoniades [*69*] suggests that "various current insulin assays determine only a fraction of the 'total' insulin activity in serum."

We have incubated fasting serums from five diabetic and five non-diabetic subjects with adipose tissue extracts according to the methods described by Antoniades and have been unable to find any increase in plasma insulin as measured by immunoassay. Since it is generally appreciated that the isolated rat diaphragm is very sensitive to composition of the incubation medium and frequently exhibits increased glucose uptake in the presence of noxious influences, it seems reasonable to suggest that glucose uptake by itself does not constitute definitive evidence of the presence of insulin in complex mixtures. Until it is demonstrated that "insulin-like activity" as measured by glucose uptake in this or any other biologic system behaves like insulin in other respects as well, and can be inhibited by potent insulin antiserums, the presence of true insulin remains unproved.

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Immunologic Aspects of Insulin*

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THE antigenicity of insulin was at one time in question [*1*]. Although insulin-sensitizing [*2–5*] and complement-fixing [*5,6*] antibodies had been demonstrated by several investigators in immunized guinea pigs, difficulty in immunization of rabbits with insulin was reported [*7,8*] and only rarely did a human subject treated with insulin present evidence of insulin immunity. Thus, in 1948 Smelo [*9*] collected a total of fifty-four cases of clinical insulin resistance from the English literature; in only ten of these cases were immune mechanisms assigned a causative role, in several because of associated insulin allergy. Until recently, evidence of insulin-antibody formation in man had only rarely been presented, usually by the demonstration of insulin-neutralizing activity in serum [*10–14*] or serum globulin fractions [*15–17*] or of insulin-sensitizing antibodies active on passive transfer (see [*11*] for review of older literature), less commonly by demonstration of "blocking" activity of serum on skin sensitivity to insulin [*13,18*].

In recent years this picture of insulin as a weak antigen has changed considerably. In 1955 Moloney and Coval [*8*] demonstrated that guinea pigs regularly produced insulin-neutralizing antibodies in response to immunization with crystalline beef or pork insulin and that these antibodies were capable of inducing diabetes by passive transfer to mice. However, in not one of thirty rabbits immunized in a similar fashion did detectable antibodies develop. At about the same time, with the aid of I^{131}-labeled insulin, insulin-binding antibodies were demonstrated in the serums of virtually all human subjects treated with insulin [*19,20*]; the absence of widespread clinical insulin resistance was attributed to low antibody concentrations in the majority of cases [*20*]. Also, Arquilla and Stavitsky [*21*], employing the technic of hemagglutination or hemolysis of insulin-sensitized red blood cells, were able to demonstrate the presence of insulin antibodies in virtually all immunized rabbits and in human subjects resistant to insulin. Furthermore, the experimental induction of diabetes by the administration of potent antiserums [*8*] has been amply confirmed and extended [*22,23*].

Since we are here concerned mainly with untoward clinical effects of insulin immunization, the remainder of this discussion will deal primarily with insulin immunity in man.

INSULIN ALLERGY

Although insulin allergy and insulin resistance may coexist [*9*], these phenomena are more frequently observed independently. Since sensitivity to contaminating substances in insulin preparations is probably much more common than true insulin sensitivity, the diagnosis of insulin allergy should be made only after careful consideration and thorough investigation. The tendency for dermal reactions (erythema, induration) to develop at sites of insulin injections is rather frequently observed shortly following institution of insulin therapy; an incidence as high as 56 per cent has been reported [*24*]. Such reactions generally disappear after a few weeks or months of continued insulin therapy but occasionally persist for longer periods. The abolition of reactions in sensitive patients by the use of repeatedly recrystallized insulin [*25*] or of commercial insulin preparations subjected to brief boiling, a procedure that does not abolish hormonal activity [*26*], suggests that insulin itself is not the dermal sensitizing factor. We have considered the possibility that this type of sensitivity may be due to subclinical immunization to beef or pork proteins as a result of lifelong dietary exposure. Farr [*27*] has, in fact, demonstrated circulating antibodies to beef serum albumin in human subjects. The gradual disappearance of dermal reactions might be explained on the basis of the development of "blocking" antibodies to these proteins, following repeated

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parenteral immunization with commercial insulin mixtures.

True insulin allergy is a much less common occurrence; its manifestations include generalized urticaria, angioneurotic edema or even anaphylactic shock. Such reactions most commonly occur shortly after resumption of insulin therapy following a period of omission, are not abolished by boiling insulin [*28*] and even follow the use of human insulin in sensitized subjects [*12*]. Allergic subjects may show exquisite sensitivity of skin and conjunctivas to minute amounts of insulin and their serums frequently contain antibodies capable of sensitizing normal skin by passive transfer [*11–13,29–31*].

Skin sensitizing antibody is thermolabile [*12*] and is recovered with the beta globulins by electrophoresis convection [*31*]. Desensitization may be associated with the appearance of "blocking" antibody capable of inhibiting the skin reaction to insulin in sensitized subjects [*13*]. Blocking antibody is heat stable and is separated with both slow and fast gamma globulins by electrophoresis convection [*18*]. Blocking antibody, insulin-neutralizing antibody and insulin-binding antibody may be identical, but because of reported differences in electrophoretic migration, the question remains unsettled.

INSULIN IMMUNITY AND INSULIN RESISTANCE

Since the blood sugar of completely depancreatized subjects is generally well controlled on 30 to 40 units of insulin daily [*32*], it might be supposed that insulin requirements in excess of these amounts must necessarily indicate some resistance to insulin. However, the validity of such a supposition is questionable, for removal of the pancreas is followed not only by the total absence of endogenous insulin but also possibly by the absence of other hormonal factors that counteract the action or effects of insulin. Although glucagon alone would not seem to be of great importance in this respect, since it does not diminish the tissue response to insulin [*33*], other factors related to normal pancreatic function might play some role, as yet unsuspected. Nevertheless, whatever factors are operative in increasing insulin requirements in diabetic patients above those reported in depancreatized subjects, the vast majority of diabetic patients require less than 80 to 100 units insulin per day. Therefore, although any dividing line must be purely arbitrary, it is reasonable to regard the persistent requirement of more than 120 to 150 units daily as indicative of abnormal resistance to insulin and to search for its causes.

The emphasis on insulin immunity in the present discussion is not meant to minimize the importance of other factors in increasing insulin requirements in certain disorders. Hormones, notably from the pituitary and adrenal cortex [*34*], various reported plasma inhibitors of insulin [*35–37*], some of which may be the pituitary or adrenal cortical hormones themselves or, at least, are produced under their influence [*35,36*], and finally, perhaps, abnormal tissue refractoriness to insulin, may all induce a state of insulin resistance. However, the known hormonal factors are rarely indictable as causative of such a degree of resistance as that under present consideration, and most cases of chronic insulin resistance, as defined by the criteria employed here, must be considered as immunologic problems. In all but one of thirty-six serums from chronically resistant diabetic subjects studied by us, the concentration of insulin-binding antibodies determined with I^{131}-labeled insulin was found to be high enough to indict insulin immunity as responsible for the resistance. (Table 1.) The exceptional case was that of a young woman who, although tolerant of 300 to 400 units insulin per day, was not readily prone to ketosis on withdrawal of insulin.

Insulin immunity can properly be considered only in relative terms since insulin-binding antibodies are demonstrable in the serums of almost all human subjects treated with insulin for an appreciable period of time. This fact was first appreciated when, in the course of studies on the metabolism and turnover of insulin-I^{131}, it was observed that insulin-treated diabetic and schizophrenic patients showed markedly prolonged retention of the labeled insulin within the circulation. This was in contrast to normal subjects or diabetic and schizophrenic patients never treated with insulin or treated for only a few weeks [*19,20*]. It was demonstrated by means of paper and starch block electrophoresis, by paper chromatography and by ultracentrifugal analysis of plasma insulin-I^{131} that the labeled insulin is bound to insulin-binding antibody in the plasma of treated subjects. In consequence, the insulin is restricted from easy transcapillary passage, whereas in non-treated persons and animals the small unbound insulin molecule is permitted readily to escape from the blood stream and to accumulate and be rapidly metabolized in certain extravascular sites,

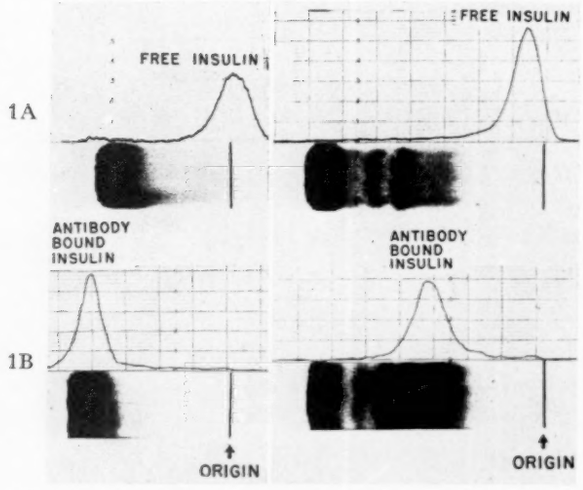


FIG. 1. A, non-immune plasma. B, immune plasma. Demonstration of insulin-binding antibody by paper electrophoresis (*right*) and chromatoelectrophoresis (*left*). In the latter procedure strips are exposed to the open atmosphere during electrophoresis for forty-five to ninety minutes so that migration of plasma proteins results primarily from hydrodynamic flow chromatography. From: BERSON, S. A. and YALOW, R. S. In: Detection and Assay of Hormones by Immunochemical Means. Ciba Foundation Colloquia on Endocrinology. Boston, 1961. Little, Brown and Co.

notably in liver and kidney, and thus exhibits a half life (after distribution equilibrium) of only thirty-five minutes in man and twenty-five minutes in the rabbit [*20*].

The presence of insulin-binding antibodies in all insulin-treated human subjects was subsequently confirmed by Skom and Talmage [*38*] who showed that rabbit anti-human serum globulin uniformly precipitated I^{131}-labeled insulin in the serums of treated subjects only, and by Gordis [*39*] who demonstrated that cold alkaline ethanol-salt solutions would precipitate insulin-I^{131} together with the serum globulins in the plasma of patients treated with insulin but not of control subjects. Furthermore, Welsh et al. [*40*] found that the insulin-neutralizing capacity of plasma was correlated with marked retention of insulin-I^{131} in the circulation of the plasma donor and confirmed that significant retention of insulin-I^{131} occurred only in treated patients.

The simplest technic for demonstration of insulin antibodies is by means of paper electrophoresis or chromatography of serum or plasma to which a minute quantity of I^{131}-labeled insulin has been added [*20*]. The tendency of insulin-I^{131} to adsorb firmly to paper [*20,41*] expedites the

TABLE 1
INSULIN-BINDING CAPACITIES OF PLASMA IN INSULIN-TREATED SUBJECTS

Patient	Insulin Dose (unit/day)	Binding Capacity (unit/L. plasma)
Non-Resistant Patients		
36 patients	<70	<10
H. L.	0 (55 units previously)	22
L. Sy.	Orinase (30 units previously)	22
J. Ga.	90	28
Ma. (acromegaly)	60	35
Insulin-Resistant Patients		
T. No.	300–400	2–5
J. Ha.	120	57
L. J.	480	60
A. P.	200	70
Sin.	120	82
E. C.	300	85
Na.	300	>100
Ja.	200	>100
E. M.	150	106
G. M. (10 yr. old)	130	110
Fr.	200	110
L. S.	190	120
Ri.	90 uncontrolled	135
Br.	180	135
Je.	300	140
Bi.	125 uncontrolled	140
D. Sh.	200	150
H. Ro.	200–400	150
B. Ro.	250	168
R. W.	500	180
Gee.	190	180
Gr.	145	195
Lu.	340	200
M. L.	150	210
Gru.	800	210
Go.	>150	240
Ge.	700	250
Wo.	120 uncontrolled	475
Si.	180	500
Ha.	500	500
Di. (22 mo. old)	54 uncontrolled	>500
J. B. (5 yr. old)	700	1,250
M. S.	4,800 units in previous 48 hours	270
Ke.	20,000 units previous week	750
Pl.	14,000 units previous day	4,700
Ni.	500	>1,500

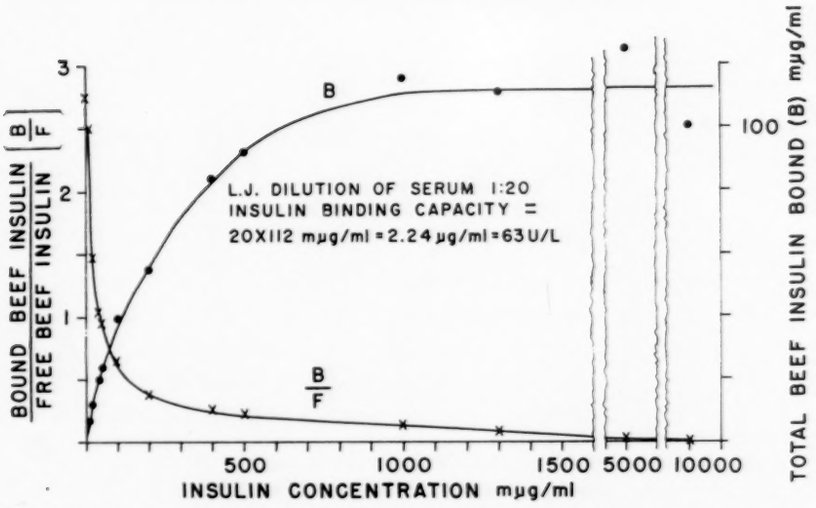


FIG. 2. Ratio of antibody-bound to free beef insulin-I^{131} (B/F) and concentration of bound beef insulin (B) as a fraction of the concentration of beef insulin in mixtures. As insulin concentration increases, bound insulin (B) approaches an asymptote (112 mμg./ml.) representing the saturation capacity of antibody.

ready identification of insulin-binding antibody since, in insulin antiserums, insulin-I^{131} is partly or completely carried along with the inter beta-gamma globulins [*20,42*], whereas in non-immune serums the insulin-I^{131} remains adsorbed to the paper at the site of application (origin). (Fig. 1.) The total binding capacity of an antiserum is determined from analysis of mixtures of appropriately diluted antiserum and insulin (including trace insulin-I^{131}) over a range of concentrations sufficient to approach saturation of antibody [*20,43–47*]. (Fig. 2.)

Only the simplest apparatus is required for chromatographic demonstration of the ratio between antibody-bound and free insulin in any mixture. A strip of Whatman 3 mm. paper is moistened with veronal buffer (ionic strength 0.025 to 0.1) and stretched horizontally across two vertical supports, one end of the strip being immersed in the buffer solution. Antiserum-insulin mixture is applied along a line close to the support at the immersed end of the strip. After fifteen to twenty minutes at room temperature the plasma proteins will have moved 2 to 3 inches away from the line of application as a result of hydrodynamic flow of buffer solution. A small amount of bromphenol blue, added to the mixture before chromatography, stains the serum albumin and aids in visualization of the migrating proteins. After drying, the strip may be assayed in an automatic strip counter, or the regions corresponding to free insulin at the origin and to antibody-bound insulin with the serum proteins may be cut out and counted separately in any suitable counter. Alternatively, the insulin-antiserum mixture may be put on a powdered cellulose column [*47*] and then washed through several times with buffer. The column method is less satisfactory than the strip technic owing to the tendency of the buffer to elute small amounts of free insulin adsorbed to the column [*48,49*] and the greater inconvenience in the preparation of columns and assay for adsorbed radioactivity.

In the majority of diabetic subjects treated with insulin, the concentration of circulating insulin-binding antibody is such that the insulin-binding capacity of the plasma is usually less than 10 units per L. plasma [*20,44–47*]. (Table I.) Since proteins in the circulating plasma volume (normally about 3 L.) account for only about 40 to 50 per cent of total exchangeable serum protein, we can calculate that the total body content of antibody is approximately equivalent to that contained in 7 L. plasma and that the *total* insulin-binding capacity of non-resistant subjects is generally less than 70 units [*20*]. In distinct contrast, the insulin-binding capacity of plasma in severe chronic insulin resistance is almost always greater than 60 units *per L.* and may reach values as high as 500 to 1,000 units *per L.* or greater [*43–48,51*]. (Table I.)

In order to relate insulin requirements to antibody concentration, we have to inquire of

the ultimate fate of antibody-bound insulin. An important consideration here is the rate of disposal of insulin-antibody complexes. In precipitating antigen-antibody systems, large antigen-antibody aggregates are removed from the blood stream very rapidly, probably by RE cells in lymph nodes and other organs. Since, however, insulin-antibody complexes are rather small [*46*] and generally do not precipitate,* even in the test tube, at high concentrations in the antibody excess range [*46*], their intravascular life span is considerably longer than that of the large aggregates formed in precipitating systems. Weigle [*53*] has found, in rabbit anti-human serum albumin and anti-human gamma globulin systems, that soluble antigen-antibody complexes, formed in the region of marked antigen excess, are eliminated from the circulation with a half life averaging about two to three days. If we assume the same for the insulin-antibody system in man we can expect that about 25 per cent of antibody-bound insulin would be disposed of daily through specific immunologic removal mechanisms and thus wasted as far as hormonal benefit is concerned. Therefore, in non-resistant subjects, even if all antibody present remained continually saturated with insulin, the additional insulin required to compensate for immunologic wastage would generally not exceed 70 units × 25 per cent/day = 17.5 units/day. For this reason increased insulin requirement due to the presence of antibody usually escapes notice. However, similar calculations based on insulin-binding capacities in resistant subjects lead to the realization that, when antibody concentration is maintained at a high level by a high rate of antibody production, immunologic wastage can account for insulin requirements of many hundreds of units daily. Not all antibody-bound insulin is destined for immunologic elimination, however, since insulin-antibody complex formation is reversible by dissociation [*20,38,44–46*], the rate of release of insulin varying from a few per cent per hour to 25 to 30 per cent per minute, depending on the characteristics of the antibody [*44–46*]. Although a certain fraction of the dissociated insulin will rebind to antibody if a significant excess of uncomplexed antibody molecules is available, some will escape into extravascular sites. Here again a fraction will bind to extravascular antibody but some will gain access to tissue cells and exert an hormonal effect or will be degraded by the "insulinase" system [*54*]. Antibody-bound insulin is not susceptible to the action of liver insulinase [*55*]. The dissociation of previously bound insulin appears to be responsible for late hypoglycemic reactions occurring repeatedly for several days after therapy with massive doses of insulin in resistant subjects [*18,44–48*].

Thus, at least three factors must be taken into consideration in the quantitative interpretation of insulin requirements in insulin resistance: (1) antibody concentration, (2) rate of elimination of insulin-antibody complexes, and (3) kinetic factors involved in complex formation, i.e., rates of formation and dissociation of complexes. Antibody concentration is readily measured by methods already described. Although there is no strict correlation between antibody concentration and insulin dosage, as can be seen from Table 1, patients taking the largest amounts of insulin generally exhibited the highest antibody concentrations. Differences in rates of immunologic elimination or in rates of complex formation and dissociation among patients with similar antibody concentrations could possibly account for two- or threefold differences in insulin requirements. However, it is perhaps unwise to equate insulin *requirements* with insulin *dosage* since in many cases adequate control was not being effected by the dosage given and in others there had been no attempt at regulation with a minimally effective dose.

NATURE OF INSULIN ANTIGENICITY

The *nature* of insulin antigenicity is not completely clear. The studies of Sanger and his co-workers [*56*], Ishihara et al. [*57*], and Nicol and Smith [*58*] have provided us with the complete amino acid sequences of beef, pork, sheep and horse insulin [*56*], sperm whale [*56,57*] and sei whale [*57*] insulin and human insulin [*58*]. Human insulin differs from all the animal insulins listed in containing threonine instead of alanine in the C-terminal position of the B chain (B 30). In addition, the various insulins may differ in one or another of residues 8, 9, 10 within the cyclic disulfide bridge of the A chain; pork, human and sperm whale insulin being identical in this region. Thus pork and sperm whale

* Positive precipitin reactions have been obtained in rare instances [*11*]. Recently Moloney and Aprile [*52*] demonstrated flocculation of insulin-antibody complexes in a single horse antiserum. Since hormonally active insulin as well as insulin antibody could be recovered from the flocculates, the phenomenon could not be attributed to contaminating proteins.

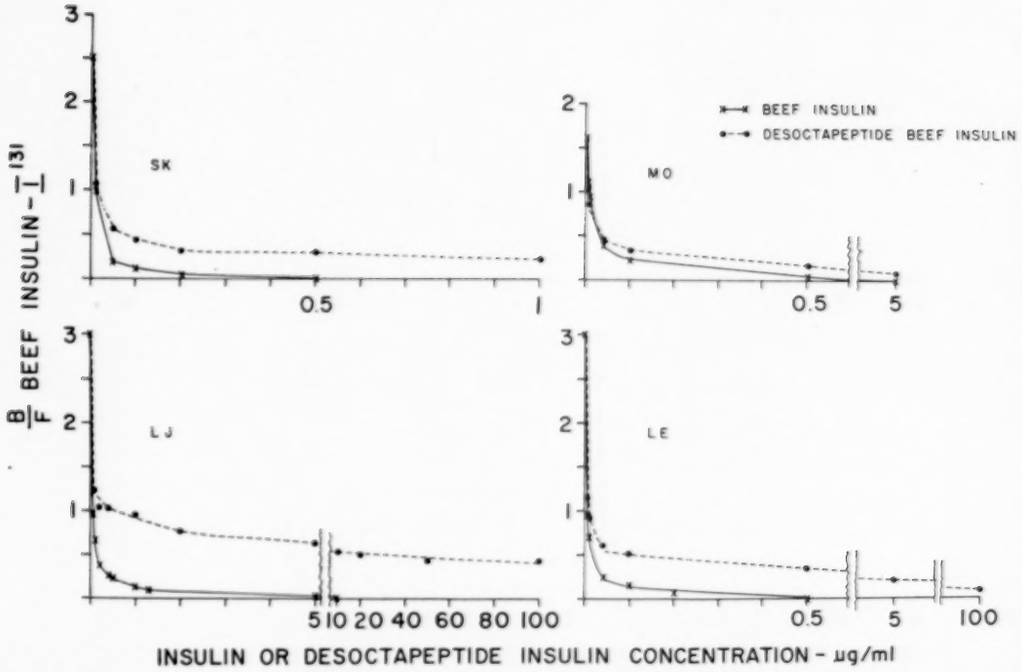


FIG. 3. Cross reactions of unlabeled beef insulin and desoctapeptide beef insulin competing against tracer concentrations of beef insulin-I^{131} in human antiserums against beef-pork insulin. Ratio of antibody-bound to free beef insulin-I^{131} as a function of unlabeled beef insulin or desoctapeptide beef insulin.

insulins are completely identical with respect to amino acid sequences and differ from human insulin only at B 30. If we assume, as a working hypothesis, that antigencity depends on some distinction in amino acid sequence only the 8–10 region of A chain and the carboxyl end of the B chain should represent potential sites of antigenicity. Experimental support for the former region has been presented [*59*]. Differences in reactivity of the four ungulate species of insulins (which differ only at A8–10) with certain human antiserums suggest that this region not only participates in the reaction with antibody but also represents a site of antigenicity. Furthermore, insulins differing in only one of the A8–10 residues reacted more alike than insulins differing in two or three of the residues [*59*]. It is not yet known whether the B chain C-terminus is antigenic in man. When compared with intact beef insulin, desoctapeptide beef insulin (lacking the residues B23–30) competes against the binding of beef insulin-I^{131} by human antiserums strongly at low concentrations but weakly at high concentrations. (Fig. 3.) These data alone might be equally compatible with a very weak reaction with one group of antibodies or with the presence of a small (0.1 to 1.0 per cent) contaminant of intact insulin.* In the latter event, the observations would be consistent with the presence of some antibody directed against the carboxyl end of the B chain with which the desoctapeptide insulin cannot react. However, the contamination, if present, would (on the basis of the experimental data) have had to have been somewhat variable from one antiserum to another. Furthermore, precisely the same sort of observations has been made with horse anti-beef insulin serum in which, in addition, the cross reaction of intact insulin with desoctapeptide insulin also is weak at high concentrations. (Fig. 4.) Since horse and beef insulin have the same B chain, there is no reason, on the basis of amino acid sequence, to expect that the carboxyl end of beef insulin B chain is antigenic in the horse. Moreover, there is experimental evidence to indicate that differences in amino acid sequence are not the sole basis for immunologic individuality. Recent studies [*61*] have shown that certain human antiserums distinguish strikingly between pork and sperm whale insulin, reportedly identical in amino acid sequences [*56,57*]. These observations

* The desoctapeptide insulin preparation employed in these studies exhibited no hormonal activity [*60*] but a small contaminant could remain undetected.

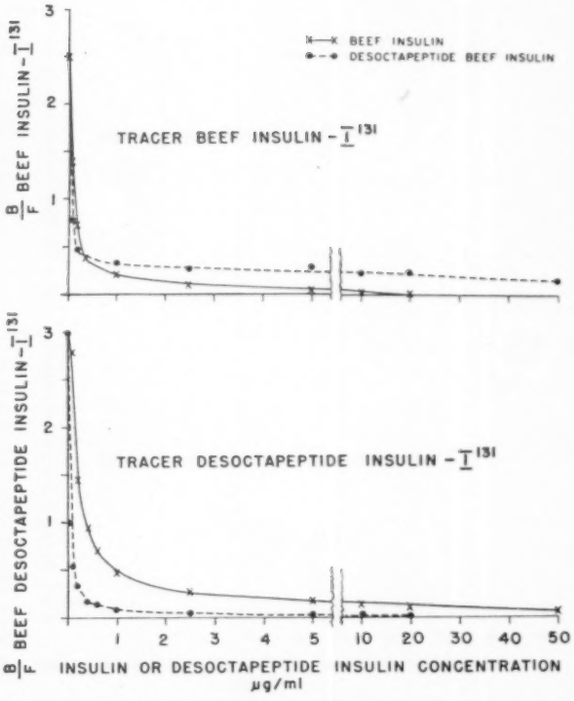


FIG. 4. Cross reactions of unlabeled beef insulin and desoctapeptide beef insulin competing against tracer concentrations of beef insulin-I^{131} (*top*) or desoctapeptide insulin-I^{131} (*bottom*) in a horse antiserum to beef insulin.

lead to the conclusion that these two insulins must differ in some structural manner and that this difference is immunochemically recognizable. So also, it is possible that changes induced in the three dimensional structure of insulin by the loss of 8 amino acids could have lessened the reactivity of a reacting site in the A chain of desoctapeptide insulin to account for the weak reaction with one group of antibodies. The importance of structural integrity of the insulin molecule is also evident from the fact that neither A chain nor B chain is reactive with insulin antibodies [*59*]. In support of these interpretations are observations that the reaction of desoctapeptide insulin at low concentrations, in some antiserums, is even stronger than that of intact beef insulin and that the difference between intact and desoctapeptide beef insulin, even at high concentrations, is still less than that between intact beef insulin and intact whale insulins in some antiserums [*62*]. Furthermore, Moloney and Coval [*8*] have concluded that, in some species, extracted insulin and endogenous insulin are immunologically distinct. One basis for this conclusion was the observation that a sheep immunized with pork insulin was immune not only to pork insulin but also to crystalline sheep insulin and yet was not diabetic, apparently responding to its own endogenous insulin. However, in view of the possibility that endogenous output could far exceed the quantity of sheep insulin administered (20 U), an *in vitro* demonstration of immunochemical distinction between extracted and endogenous insulin would be more convincing. The demonstration of reaction of endogenous human plasma insulin as well as of extracted human insulin with guinea pig antiserums [*49*] and the ease of inducing an acute diabetic syndrome, characteristic of "totally" depancreatized animals [*63*] in mice [*8*], rats, rabbits and cats [*63*] by the injection of potent antiserums indicate that, in many species, endogenous insulin reacts with heterologous insulin antibodies and that *all* hormonally active endogenous insulin can be neutralized by this means.

Moloney and Coval [*8*] found that five of six batches of guinea pig insulin injected into mice could not be neutralized by sheep anti-pork insulin serum or by guinea pig anti-beef or anti-pork insulin serums capable of neutralizing a variety of other animal insulins. It may be that guinea pig insulin differs uniquely in its chemical structure from other mammalian insulins and so exhibits a distinct immunologic specificity.

TREATMENT OF INSULIN RESISTANCE

The treatment of insulin resistance can be discussed only briefly. Since infection is not infrequently an immediate precursor to a sudden rise in insulin requirements, possibly by providing a non-specific stimulus to antibody production [*11*], occult sources of infection should be thoroughly investigated. In the absence of infection, corticosteroid therapy should be given a trial for at least two weeks. Favorable responses have been reported by a number of workers [*14,17, 64–68*]. From the rate of improvement in responsive cases it is surmised that the effect is manifested through inhibition of antibody production rather than through influence on the insulin-antibody reaction itself. In about half the cases with which we have been familiar, corticosteroids alone induced a significant fall in insulin requirements and in two of these cases Morse [*68*] has demonstrated a fall in concentration of insulin-binding antibody. A decrease in concentration of passive transfer antibodies was observed by Oakley et al. [*67*] in three resistant

patients who responded well to prednisone therapy. An occasional patient may benefit perceptibly from the addition of one of the newer hypoglycemic agents such as tolbutamide or phenethylbiguanide (DBI). Since these drugs enhance endogenous insulin when insulin reserve exists, a good therapeutic response may indicate that the patient is deriving relatively greater benefit from his own insulin than from the exogenous animal insulins. This would not be unexpected since the antibody generally has a greater affinity for the beef and pork insulins present in commercial preparations than for human insulin; thus at the same insulin concentration a smaller fraction of human insulin is bound [*50*]. Lowell [*12*] has reported a lowering of blood sugar following administration of human insulin to an insulin-resistant patient who failed to respond to a similar dose of beef insulin. The partial species-specificity of the insulin-antibody reaction suggests trial of other insulins. Since the order of reaction of various insulins with several human antiserums was found to be beef $\cong$ sheep $\gg$ pork $>$ horse [*59*], the substitution of horse insulin, if available, or of pure pork insulin, now commercially available, is indicated in otherwise refractory cases. However, since Lilly insulin contains 30 to 70 per cent pork insulin, striking results cannot be expected from pure pork insulin if there is marked resistance to beef-pork insulin mixtures. In any event, insulin therapy should be pursued to the fullest extent necessary to control ketosis since no advantage accrues from its withdrawal. Despite all efforts, resistance remains obstinate in some patients and continued administration of large doses of insulin is necessary. In such cases, insulin U-500 will permit injection of smaller volumes.

To end on a note of optimism, it may be observed that insulin antibodies have been exploited to good use in the immunoassay of insulin [*49*], in the study of islet cell morphology by the fluorescent-antibody technic [*69,70*] and in the experimental production of insulin-deficiency states [*63*].

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The Production of Experimental Diabetes by Means of Insulin Antibodies*

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SEVERAL methods have been described for the production of diabetic syndromes in experimental animals [1]. They involve the injury, destruction or removal of the insulin-secreting cells of the pancreas [2] and result in syndromes characterized by hyperglycaemia, glycosuria and faulty carbohydrate metabolism [3]. The aetiology of diabetes in man is still uncertain [4] but the experimental methods have been designed to produce and have resulted in the production of syndromes in laboratory animals which are due to relative or absolute insulin deficiency. As such, however, they have certain disadvantages. First, they are technically difficult or unpredictable in their effects and, secondly, they result in lesions affecting cells other than those which synthesise and secrete insulin. Thus total ablation of the pancreas, although a relatively simple procedure in such animals as the cat and dog, is technically difficult or impossible in small animals, such as the rat [5]. Alloxan, by contrast, is easy to administer but its effects, comparable with those of partial pancreatectomy [6], are unpredictable. With the loss of cells necessary for the normal digestion and absorption of food, and of cells in the islets whose functions are uncertain, the pancreatectomised animal presents a difficult nutritional problem even when receiving adequate doses of insulin [5,7]. Extrapancreatic lesions in the animal given alloxan are said to be "minor in character from the morphologic point of view [8]," but they occur [6] and their metabolic significance is unknown. Permanent diabetes accompanied by lysis or disappearance of the beta cells [1] has also been produced by more indirect methods but the exciting agents (growth hormone, adrenocorticotrophic hormone and cortisone) have to be administered for long periods and have actions, often of unknown nature, upon cells elsewhere in the body. It is reasonable to conclude, therefore, that the syndromes produced by these methods are not solely due to insulin deficiency.

A more specific method was suggested by observations reported by Moloney and his coworkers [9,10], who showed that the intraperitoneal injection of guinea pig anti-insulin serum into mice causes hyperglycaemia, glycosuria, ketonuria and loss of body weight. Such serum inhibits the stimulant effect of insulin upon biologic processes in the isolated rat diaphragm [11] and rat epididymal fat pad [12,13]; reduces or abolishes the hypoglycaemic effect of insulin in mice [9] and rats [14]; binds I^{131}-labeled insulin in the globulin fraction of the serum proteins [15,16]; agglutinates insulin-conjugated sheep red cells [14] and stimulates phagocytosis of insulin-coated bentonite particles [17]. These properties of guinea pig anti-insulin serum and similar effects produced by serum of other insulin-treated animals suggest that the active components are insulin antibodies [18,19]. These antibodies are not strictly species-specific, since serum obtained from animals treated with insulin from one animal source will react with insulin obtained from others [9,11,14,20]. The antibody produced by the guinea pig differs from those of the horse [10,17] and sheep [17] for it induces transient hyperglycaemia on intravenous injection into the rat, rabbit, mouse, sheep, pig and dog [9,21,22]. Thus it not only combines with insulin extracted from the pancreas but also appears to inactivate endogenously secreted insulin. These observations suggested that guinea pig anti-insulin

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serum would provoke in experimental animals a diabetic syndrome specifically due to insulin deficiency.

GUINEA PIG ANTI-INSULIN SERUM

The guinea pig is the only animal known at present which, after prolonged treatment with insulin, will yield serum capable of inducing hyperglycaemia in experimental animals. Small amounts have been obtained from individual animals for experiments carried out *in vitro* [*11,13,16*], but larger quantities of potent serum are needed *in vivo*. The highest yields of active serum have been obtained by giving monthly injections of insulin in an oily emulsion and bleeding by cardiac puncture two weeks after the third and each subsequent injection [*14*]. The numerous deaths due to hypoglycaemia reported by Moloney and Coval [*9*] can be avoided by using a heavy (Liquid paraffin, B.P.; Liquid Petrolatum, U.S.P.) instead of a light (Bayol F, B.P. range 274, 380°c., Imperial Oil Co.) mineral oil in the preparation of the emulsion. The viscosity of the oil and not, as was previously suggested [*14*], the nature of the emulsifying agent appears to determine the rate of absorption of insulin from the site of subcutaneous injection; for emulsification, mannide mono-oleate (Arlacel A, Atlas Powder Co., Delaware) and lanolin (Adeps Lanae, anhydrous, B.P., U.S.P.) have been used. In this way about 5 ml. of serum can be obtained every month from each guinea pig and will neutralise 1 to 3 units insulin/ml. serum. One initial injection of an emulsion containing Mycobacterium butyricum [*14*] may result in the production of more active serum. Assay of the harvested serum in rats [*14,21*] has the advantage over *in vitro* methods, such as that of Yalow and Berson [*15,16*], in that it gives an estimate of the capacity of the serum to inhibit hormonal activity in the intact animal. It should be emphasised, however, that this method of assay is crude.

Some Effects of Guinea-pig Anti-insulin Serum in Experimental Animals. To induce hyperglycaemia in an animal, the active serum should be injected intravenously or intraperitoneally; subcutaneous injections have little or no effect. For this reason, three methods of serum administration have been used, the choice depending upon the effect required.

1. *Single intravenous injection:* This rapidly induces hyperglycaemia of relatively short duration; the effects produced in rats, rabbits, a cat, pig, sheep and dogs have already been reported [*21,22*]. Immediately following the injection, the blood sugar concentration begins to rise, the initial rate of increase depending upon at least three factors: (1) The animal species. In conscious well nourished animals, the highest rate of increase (3.16 mg./100 ml./minute) was observed in the rat and the lowest in the sheep (0.67 mg./100 ml./minute), with intermediate values in the dog and rabbit. (2) Nutritional state. Prior fasting of the conscious rat for twenty-four hours reduces the rate of increase to 1.38 mg./100 ml./minute. In conscious dogs [*23*] the highest rates of increase in plasma sugar concentration were found in active well nourished animals (1.3 to 1.5 mg./100 ml./minute) whilst rises of less than 1 mg./100 ml./minute occurred in dogs in poor physical condition. Two dogs which did not respond to the serum injection at all died several hours later and were found to have had canine distemper. (3) Anaesthesia. The anaesthesia of well nourished conscious rats has no appreciable effect upon the initial rate of increase in blood sugar concentration, but the rate (0.27 mg./100 ml./minute) found in fasted anaesthetised rats is much less than that found in conscious fasted rats. No rise was found in one anaesthetised dog which had been deprived of food for at least two days [*22*].

The initial rate of increase in blood sugar concentration provoked in the rat is independent of the dose of serum injected [*21*], but dosage does determine the duration of the hyperglycaemic response [*14*]. A dose of serum which would produce hyperglycaemia in the conscious rat lasting about an hour (i.e., sufficient to neutralise about 2.5 units insulin/kg.), has a much more prolonged effect (up to six hours) in the conscious rabbit, dog and sheep. The maximum blood sugar concentrations which have been found in rats given such injections (260 to 360 mg./100 ml.) have usually been higher than those found in dogs (200 to 250), rabbits (200 to 300), a cat (250), pig (200) and a sheep (220 mg./100 ml.); these figures refer to the maxima found in all experiments irrespective of the state of nutrition or consciousness of the animals.

2. *Continuous intravenous infusion:* This method has been used in rats [*24*] to induce a diabetic state (*vide infra*) and to determine the ability of the rat to store and secrete endogenous insulin. When the antibody is given as an intravenous

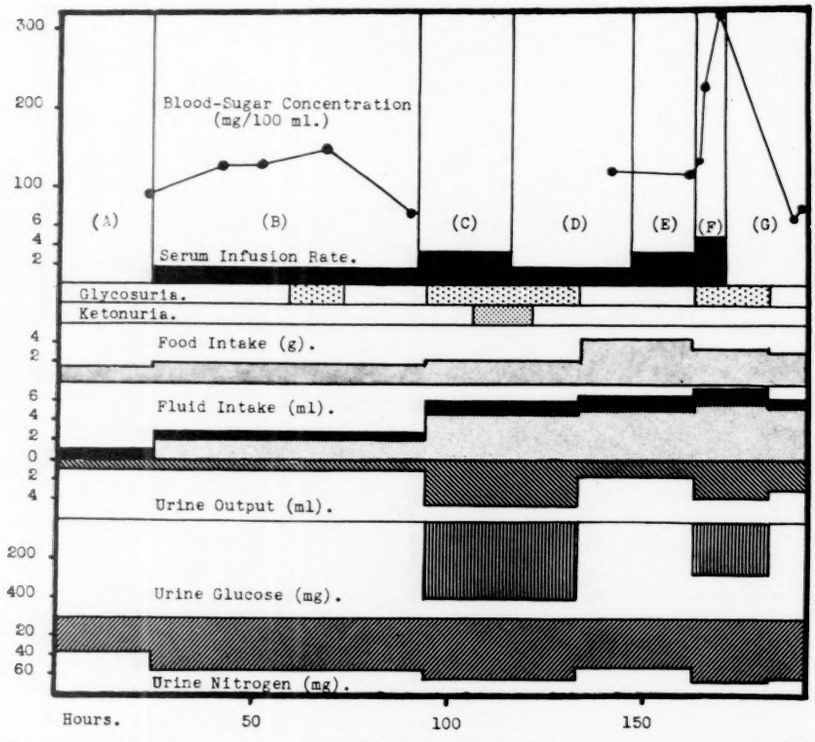


FIG. 1. Continuous intravenous infusion of anti-insulin serum given to rat. All intake and output rates stated per kilogram initial body weight per hour. The black area in the fluid intake represents the fluid administered intravascularly, the cross hatched area the water taken orally. The serum infusion rate gives the number of units of insulin neutralised by the infused serum per kilogram initial body weight per hour; the periods of infusion referred to in the text are shown (A to G). Glycosuria and ketonuria were detected using Clinistix® papers (active principle, glucose oxidase; Ames, Indiana) and Acetest® tablets (active principle, sodium nitroprusside; Ames, Indiana), respectively, as previously described [*24*].

infusion to the conscious rat at a rate sufficient to neutralise more than 4.4 units insulin/kg. body weight per hour, the blood sugar concentration rises steadily to about 300 mg./100 ml. at a rate comparable with that following the injection of a single dose. Slower infusions produce an initial transient rise which is halted for an hour or two before continuing to a level determined by the rate of the infusion. The effects of more prolonged infusions at varying rates are illustrated in the results of the following experiment:

Continuous intravenous infusion of anti-insulin serum for six days: Using the method previously described [*24*] and a fraction collector for hourly urine sampling, a continuous infusion of anti-insulin serum (Batch 6,884; 1 ml. neutralised approximately 3.6 units insulin) was given for 146 hours after a control period lasting twenty-four hours from the time of insertion of the catheters. The rates of serum infusion, food and total fluid intake, and of urine and urinary nitrogen and glucose excretion are shown in Figure 1. No samples of blood were obtained from 90 to 180 hours due to temporary blockage of the arterial catheter. Throughout the experiment the animal, an albino Wistar rat (280 gm.), behaved normally although it lost 10 per cent of its original body weight. At necropsy the liver, kidneys, stomach and other tissues appeared normal and there was no evidence of infection.

During the first period of serum infusion (Fig. 1, B, sixty-eight hours), when the animal was receiving sufficient antibody to neutralise about 1.6 units insulin/kg. body weight per hour, the blood sugar concentration rose slightly and for fourteen hours glucose appeared transiently in the urine (1.2 per cent). Within two hours of doubling the serum infusion rate (Fig. 1, C, twenty-four hours), heavy glycosuria (0.41 gm./kg./hour) and polyuria (4.5 ml./kg./hour) commenced and after fourteen hours ketone bodies were detected in the urine. With restoration of the serum infusion rate to its lower initial value (Fig. 1, D, thirty hours), the rates of glycosuria and polyuria decreased, ketone bodies and glucose disappearing

from the urine after sixteen and thirty-nine hours, respectively. Towards the end of this period of glycosuria the rat was drinking water at a very high rate (7.3 ml./kg./hour). Polydipsia continued during the subsequent aglycosuric interval and the animal began to eat more than usual (4.2 gm./kg./hour). Glycosuria and polyuria did not occur when the serum infusion rate was again doubled (Fig. 1, E, sixteen hours) but a further increase (Fig. 1, F, eight hours), to a rate neutralising 4.9 units insulin/kg./hour, caused the prompt onset of hyperglycaemia, polyuria and heavy glycosuria. When the serum infusion was stopped (Fig. 1, G, twenty-one hours), the rat had received sufficient antibody to neutralise about 300 units insulin/kg. body weight over 146 hours, but within sixteen hours the blood sugar concentration had fallen to 58 mg./100 ml. It was still consuming food and water at a high rate when it was killed.

Throughout the period of serum infusion the urinary nitrogen excretion rate remained fairly constant (48.5 to 63.9 mg./kg./hour) and higher than the rate observed during the initial control period (35.7 mg./kg./hour). However, relative to the amount of food eaten, nitrogen excretion was highest (29.8 mg. nitrogen per gram of food) during the first period of heavy glycosuria and lowest (11.6 mg. nitrogen per gram of food) during the subsequent aglycosuric interval; at other times the value ranged between 18.4 and 23.8 mg. nitrogen per gram of food.

As a method for the study of the diabetic syndrome in small animals, this is not as convenient as the one about to be described, since only a few animals can usually be used at any one time. It can be employed, as the experiment illustrates, to control the degree of insulin deficiency in an animal.

TABLE I
METABOLIC CHANGES IN RATS GIVEN REPEATED INJECTIONS OF GUINEA PIG ANTI-INSULIN SERUM (I.P.)

Data	Control Animals (Fed)	Control Animals (Fasted)	Diabetic Animals
Intake			
Food (gm.)	3.75	0	0.88
Water (ml.)	5.75	2.50	4.70
Output			
Urine (ml.)	2.33	2.12	4.38
Nitrogen (mg.)	48	38	67
Potassium (mEq.)	0.50	0.17	0.33
Glucose (gm.)	0	0	0.50
Weight loss at death (%)	0	22.	19
Blood sugar at death (mg./100 ml.)	81	64	365
Liver weight (gm./kg. body weight)	43.6	25.5	25.6
Liver nitrogen (gm./kg. body weight)	12.9	9.2	8.6
Glucose-6 phosphatase (mg. phosphorus/kg. body weight/30 minutes)	608	540	1,010

NOTE: The mean values refer to nine fed and two fasted control animals and four diabetic rats observed over sixty hours, except for the nitrogen and potassium excretion rates which refer to mean values obtained over forty-eight hours with four animals in each group. All intake and output rates are stated per kilogram initial body weight per hour.

3. *Intraperitoneal injection:* When serum is administered intravenously to small animals only small volumes can be given as an injection at one time; rats were never given more than 1.5 ml. serum at a time although this dose was repeated without undesirable effects after one hour. A single dose given intraperitoneally to the rat produces almost the same effect on the blood sugar concentration as the same dose given intravenously [*24*]. Relatively large doses can be given and the injections can be repeated. Thus it is possible to induce and maintain many rats in a diabetic state and to study the behavior *in vitro* of tissues removed from them. Its only disadvantage is that injected serum may enter the intestine instead of the peritoneum. If the dose is sufficient, injected serum induces a diabetic syndrome within a few hours. The responses evoked in rats by single injections have already been reported [*24*] but are illustrated in the following experiment in which repeated intraperitoneal injections were administered over a period of sixty hours [*25*].

Repeated intraperitoneal injections of anti-insulin serum in rats: Male albino rats (200 gm.) were given repeated (12 hourly) injections of anti-insulin serum; control animals received normal serum or saline solution at the same times and were either fasted or, like the test animals, allowed free access to food. The results of significance to this account are summarised in Table I.

Test (diabetic) rats received a total of 13.5 to 16.5 ml. serum, sufficient to neutralise between 282 and 345 units insulin/kg. body weight, over a period of sixty hours. They became glycosuric in three to five hours and ketonuric in five to twenty-three hours and remained so until they were killed by decapitation. No blood samples were drawn during life but at death they were all hyperglycaemic. They lost body weight at about the same rate as the fasted control rats; ate much less food and drank slightly less water than the fed animals; developed heavy glycosuria and marked polyuria and, relative to the

amount of food eaten, lost more potassium and nitrogen in the urine than the fed control rats. Just before being killed they showed signs of slight weakness but otherwise behaved normally. At necropsy their tissues were not pale or dry; their livers were about the same size as those of the fasted rats, and their stomachs, unlike their intestines, were distended with food. Liver glucose-6-phosphatase activity was elevated.

The Diabetic Syndrome Induced by Anti-insulin Serum. Long [*26*] has stated that the syndrome produced by total pancreatectomy of the cat is characterised, *inter alia*, by (1) hyperglycaemia and glycosuria that persist during fasting; (2) nearly quantitative excretion of ingested glucose; (3) increased urinary excretion of nitrogen both in fed and fasted animals; (4) a marked increase in acetone body content of both blood and urine, largely a consequence of the increased proportion of fat undergoing metabolism; and (5) the final development of severe acidosis, coma and death. Essentially these are the features of the syndrome induced in rats by means of guinea pig anti-insulin serum.

The changes in *blood sugar concentration* which follow the administration of guinea pig anti-insulin serum have already been described. It is important to emphasize, however, that the effect of the serum is rapid and that if sufficient is administered initially the concentration rises to diabetic levels within a matter of hours. If injections are repeated or infusions continued, the blood sugar concentration of the rat remains at about 250 to 350 mg./100 ml. as long as polyuria persists. Higher concentrations have been found only in association with oliguria, probably due to dehydration [*24*]. Within a few hours most rats stop eating but their blood sugar levels remain elevated and do not fall unless the effects of the serum are passing off.

Once the blood sugar concentration exceeds about 200 mg./100 ml., *glycosuria* begins and persists as long as the animal maintains this degree of hyperglycaemia [*24*]. Rates of glucose excretion of the order of 7 to 13 gm./kg./day have been observed, especially during prolonged periods of hyperglycaemia. The concentration of the glucose (measured as reducing substance) in the urine often reaches but seldom exceeds 10 per cent. When serum is infused at a slow rate, as shown in Figure 1 (period B), transient glycosuria unaccompanied by polyuria may be detectable qualitatively but may not be appreciable quantitatively. Fasting does not prevent glycosuria, for one rat, previously described (rat G [*24*]), continued to excrete glucose for thirty hours at a rate of 4 to 8 gm./kg./day although it ate no food during this time.

The *urinary excretion* rates of nitrogen of fasted diabetic rats have not been studied but, as illustrated by the experiments described, nitrogen excretion relative to the amount of food eaten increases during prolonged hyperglycaemia (Table 1) and falls to a low level during recovery. (Fig. 1.) This suggests that this diabetic state is accompanied by excessive breakdown of proteins in the body.

No quantitative estimations of *ketone bodies* in the blood and urine have yet been attempted but ketonuria has always been detected in animals in which persistent hyperglycaemia has developed. It was reported previously [*24*] that ketonuria begins less than five hours after the onset of glycosuria, but in later experiments two to twenty-three hours (mean, 9.4) elapsed before ketonuria was detected [*25*]. The reason for this discrepancy is not known, but it may be significant that the latter animals were fed on a diet containing more protein and were not submitted to the stress of repeated bleeding. That the appearance of the ketone bodies is associated with abnormal mobilisation of fat has been shown in rats examined during the first twenty-four hours following single intraperitoneal injections of anti-insulin serum [*27*]. At twenty-four hours the total lipid content of the liver (430 mg./100 gm. body weight) is more than twice that of control fed or fasted animals (183 and 158 mg./100 gm. body weight, respectively). The progressive rise in liver lipids, detectable after only three hours, is not due to any accumulation of phospholipids or cholesterol but to an absolute increase in the triglycerides from 124 to 367 mg./100 gm. body weight. Within three hours the concentration of non-esterified fatty acids in the serum rises from 0.22 to 1.29 mEq./L. and reaches 2.36 mEq./L. at twenty-four hours.

In only one rat (rat E [*24*]) has *coma* been reported and in no case has blood been examined to establish the presence of *acidosis*. However, oliguria, weakness, an increasing blood sugar concentration and ultimately rapid respirations suggestive of acidosis in the presence of ketonuria, have been reported [*24*].) These signs did not appear in the rats used in the recent experiments (Table 1) even after sixty hours. These animals were not bled during life, a stress which may

have precipitated the onset of renal insufficiency and acidosis in previous experiments. It is not possible to state how long rats could survive if hyperglycaemia persisted, since they have usually been killed before death occurred naturally. However, some have appeared on the point of death after periods ranging from thirty to seventy hours when repeated blood samples were being taken, and others (Table 1) were still active after sixty hours although they were slightly weak. Probably, therefore, a survival period of about three to four days is possible if the animal is not submitted to any added stress.

To these observations should be added others which are comparable with those made previously in other forms of experimental diabetes. Thus rats treated with anti-insulin serum [*24*] have distended stomachs despite the fact that they may not have eaten for many hours. In recent experiments, rats killed after twenty-four to sixty hours showed similar gastric distention [*25*]. They had been eating and their stomachs, which contained food, were of about the same volume as those of the fed control animals. Their intestines were empty, however, whilst those of the fed control animals were filled normally. This suggested that the motility of the stomach muscles of the diabetic animal is abnormal, possibly due to excessive losses of potassium in the urine. Over sixty hours the weight of the liver, relative to the body weight before injection of the serum, falls to the same extent in diabetic and fasted control animals (Table 1); the same applies to the liver nitrogen contents. This has to be taken into account when interpreting observations in enzyme studies with tissue removed from such animals. Several liver enzymes have been studied [*25*], but the first to show any appreciable change in activity is glucose-6-phosphatase. As shown in Table 1 the glucose-6-phosphatase activities of the livers of fed and fasted control rats are about the same and much less than those of rats which have been diabetic for sixty hours. Despite loss of proteins, therefore, the livers of diabetic rats show higher glucose-6-phosphatase activity than those of similarly depleted fasted animals. These findings can be compared with the gastric distention seen in "totally" pancreatectomised rats [*5*] and the elevated glucose-6-phosphatase activity seen in livers removed from alloxan-diabetic rats four or more hours after withdrawal of insulin treatment [*28,29*].

COMMENT

The parenteral administration of guinea pig anti-insulin serum has certain advantages over other methods for the induction of experimental diabetes. First, the provoking agent appears to be a specific insulin antagonist which will bind and inactivate endogenously secreted hormone. Secondly, the diabetic syndrome, in the rat at least, develops rapidly and without evidence of undesirable side effects; in other animals the blood sugar concentration begins to rise as soon as the serum has been injected. Thirdly, it is reversible, for the treated animal will recover without specific treatment or after administration of exogenous insulin. Lastly, the serum is effective in animals which are readily available in the average laboratory, is easy to administer and can be used to compare the syndromes evoked in several animal species. Its disadvantages include the difficulties involved in the production of large amounts of highly active serum and the presence in that serum of non-specific proteins.

The serum of guinea pigs treated with bovine insulin, the serum used in all the experiments herein referred to, will combine with insulin extracted from pancreatic tissue of a number of animal species [*9,11,15,18,19*]. It probably combines with insulin secreted endogenously by a number of experimental animals [*22*] but it is not known how this insulin inactivation occurs *in vivo*. Human plasma loses its insulin-like activity after it has passed through a cationic exchange resin [*30*]; after extraction from the pancreas, insulin is not absorbed by the resin under similar circumstances. This and later findings led Antoniades and his co-workers [*31,32*] to suggest that insulin can exist in the blood in two forms: a free form, which stimulates glucose consumption by the isolated rat diaphragm; and a bound form, conjugated with a basic protein, which stimulates metabolism of the rat epididymal fat pad but has no effect upon the rat diaphragm unless it is first exposed to an alkaline solution (pH, 9.8) or treated with an extract of rat adipose tissue. Guinea pig anti-insulin serum inhibits the biologic activity of the "free" form but not that of the "bound" form [*31*]. They have also suggested that the relative proportions of the two forms in the circulation may vary, the highest concentrations of "free" insulin being present when the blood sugar concentration is high [*33*]. There is also evidence that much of the

insulin in the pancreas exists in a form similar to that of the circulating "bound" hormone [*12*]. These observations still require confirmation but, whatever the nature of insulin in the pancreas and in blood, there is no doubt that insulin in human blood will combine with guinea pig anti-insulin serum *in vitro;* Yalow and Berson [*16*] have used this anti-insulin serum to estimate insulin in plasma. What is at issue is the site of action of the serum *in vivo*. Does it, for example, react with the hormone during transport in the blood from the pancreas to the tissues which require it and, if so, does it inactivate all of it or only that part which is "free"? Soon after the injection of anti-insulin serum, the plasma of the sheep and pig fails to stimulate glucose consumption by the isolated rat diaphragm [*22*]. From this it could be concluded that only the "free" form is inactivated. However, large amounts of insulin in a biologically active form can be extracted from this same plasma with acid-alcohol, which could imply that the serum has inactivated all the hormone in the plasma by firmly combining with it. Before either of these rather tentative conclusions can be substantiated, however, much more must be learned about the nature of endogenously secreted insulin, the form or forms in which it circulates in the blood and their reactions with the active component of guinea pig anti-insulin serum.

The rapidity of onset of the effects of injected anti-insulin serum has already been emphasized. This, and the absence of undesirable side effects, give this method definite advantages over the others which have been used to induce experimental diabetes. The "permanent" diabetic state induced in rats with the administration of alloxan, for example, is preceded by transient periods of hypoglycaemia and hyperglycaemia [*6*]. A more rapid onset is achieved by removal of 99.5 per cent of the rat's pancreas, for within two hours the glucose, ketone body and total lipid contents of the blood are elevated [*5,34*]; but in nearly half of the animals treated biliary obstruction develops. Withdrawal of insulin therapy from diabetic animals offers an alternative approach but in the case of the rat given alloxan, adequate control with insulin is difficult [*35*], and a significant rise in blood sugar concentration is not observed for six and a half hours [*28*]. In addition, it cannot be said that diabetic animals maintained with insulin are metabolically normal; the rats given alloxan [*28*] had blood sugar concentrations of about 40 mg./100 ml. By contrast, animals given an injection of anti-insulin serum may be perfectly normal before injection, if that is desired, and the effects of the serum may be detected within minutes. Using animals given such injections, therefore, it should be possible to distinguish the primary from the secondary effects of insulin deficiency. By inference it may then be possible to determine more about the mode of action of the hormone itself.

The reversible nature of the diabetic state induced by anti-insulin serum facilitates direct estimation of the amount of insulin stored in the pancreas of the rat and the rate at which the hormone can be secreted endogenously. Slowly infused serum causes a biphasic increase in the blood sugar concentration of the rat [*24*], but if the rate is too great the level rises progressively to 300 mg./100 ml. or more. The halt in the initial rise has been attributed to release of insulin stored in the pancreas. In support of this it has been shown [*36*] that five hours after the administration of serum to rats, little or no insulin can be recovered from the pancreas and few granules can be found in the beta cells. Also, the amount of insulin which can be extracted from the rat pancreas (5.8 to 8.4 units/kg. [*37*]) is the same as the estimate based upon the effects produced by the infused serum (5 to 8 units/kg. [*24*]). The blood sugar concentrations reached after infusion for twenty hours and the changes which occurred after the infusions were stopped suggest that the rat is able to synthesise and secrete the hormone endogenously at a rate of 1.2 to 2.6 units/kg./hour. The rat pancreas may be able to synthesise and secrete more than this, for in the present experiment (Fig. 1) diabetic symptoms appeared in the final stages of the infusion only when antibody was administered at a rate sufficient to neutralise 4.9 units/kg./hour. Moreover, there was no evidence in this experiment that the islet tissue was failing after six days; hypoglycaemia ensued when the infusion was stopped despite the fact that the animal was eating at an increased rate. Further investigation is necessary to test these conclusions, but it does seem that the endogenous insulin secreted by the pancreas can be titrated with infused antibody by using the metabolic changes in the infused animal as indicators. This is possible, however, only because the diabetic state is reversible.

Since the antibody in guinea pig anti-insulin serum is not species-specific with respect to

endogenously secreted insulin, it could be used to compare diabetic syndromes in various animals. For example it provokes hyperglycaemia in the pig, dog and cat in which different diabetic syndromes develop after pancreatectomy [*38,39*]. The technic would not require great skill but would involve the use of large amounts of serum. An alternative animal to the guinea pig should therefore be sought as a source of antibodies in high concentration capable of neutralising endogenous insulin. No such animal has yet been found but it has been suggested that the capybara be tried [*14*]; it is a much larger animal and is closely related to the guinea pig. The presence of non-specific proteins in serum offers another possible disadvantage which has not received much attention. The cat, for example, reacts strongly but transiently to the injection of guinea pig serum. Such reactions were not seen in other animals receiving their first dose, but the rabbit became sensitised after one injection and the rat after four injections administered at weekly intervals [*21*]. Non-specific reactions to serum may be seen, therefore, in animals which are hypersensitive to foreign proteins or which receive multiple injections.

SUMMARY AND CONCLUSIONS

A method has been devised for the production of experimental diabetes which is thought to be due to uncomplicated insulin deficiency and is reversible. By intravascular or intraperitoneal injection or infusion of serum from guinea pigs treated with insulin over a long period, a rise in blood sugar concentration can be produced within minutes in the rat, cat, rabbit, dog, sheep and pig. If sufficient serum is given to the rat, polyuria, heavy glycosuria and ketonuria begin and a syndrome similar to that described in the pancreatectomised rat, cat and dog develops in the animal. Following single injections of serum in doses sufficient to maintain hyperglycaemia, insulin-like activity disappears from the blood (sheep); lipids accumulate in the blood and liver (rat); and granules disappear from the beta cells and the insulin content of the pancreas falls (rat). These and other metabolic changes can be detected within a few hours. By constant infusion of serum into the rat, the insulin stored in the pancreas has been assayed and the rate at which the pancreas is able to synthesise and secrete the hormone has been determined.

It has been emphasised that more information is required about the metabolic changes produced by the anti-insulin serum *in vivo* and the chronologic order in which they occur. More should be learned also about the immunologic aspects of the reaction between the antibody in the injected serum and endogenously secreted insulin; the value of the method depends at the moment on the assumption that the antibody deprives the injected animal of insulin by combining with any which its pancreas may secrete. There is no doubt, however, that this serum does induce a diabetic state, and there is indirect evidence to indicate that the animals given such injections become insulin-deficient. It seems likely, therefore, that this method for the production of experimental diabetes, technically simple as it is, will find a variety of uses and may provide useful information concerning the mode of action of insulin and the metabolic abnormalities which are due to insulin deprivation.

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On Some Biochemical Aspects of Diabetes Mellitus*

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PATIENTS whose flesh melted away and was "sieved" through the kidneys as prodigious quantities of urine were aptly named "diabetics." When it was discovered that their urine contained large amounts of sugar a specifying adjective was added. Hence the term "diabetes mellitus." For over 200 years now the bulk of clinical observations and of experimental approaches has centered upon the derangements of carbohydrate levels and turnover in this disease. The seemingly simple query: "Why glycosuria?" has had and continues to exert a tremendous impact on the development of knowledge in the fields of nutrition, enzyme chemistry and endocrinology. In fact, only the problems of beer manufacture (how does yeast make alcohol from sugar?) and those of muscular work (how is food energy converted to mechanical energy?) have had effects on the acquisition of knowledge of cellular metabolism comparable to those stimulated by the problems of the diabetic patient. The most fruitful of the achievements of the past was of course the discovery and therapeutic application of insulin, just forty years ago.

The prolongation of life of all diabetic subjects, and especially of the ever increasing number of young adult diabetic subjects, whom insulin helped to attain the period of procreation has revealed many aspects of the disease which are difficult if not impossible to relate to our present concepts of the metabolic disturbance proper. One such set of phenomena are those which tend to appear prior to any overt significant disturbance in blood sugar regulation. All such deviations are at present said to form the "prediabetic" state [*1,2*]. The other set of clinical and pathologic changes are the specific angiopathies and neuropathies. The initial stages of these disturbances may be found during the "prediabetic" phase [*3,4*], but their full clinical expression is generally delayed for some years after overt hyperglycemia has been established.

Naunyn's opinion as to the hereditary nature of diabetes has been amply substantiated by many studies [*5–7*]. The nature of the gene change is of course totally unknown but it is clear that a person with diabetes begins life with one or more altered genes. He therefore manufactures one or more altered proteins. In due time the activities of these substances find expression in diverse ways: (1) obesity (perhaps of a particular type), (2) increased birthweight, (3) increased incidence of fetal abnormalities and higher perinatal death rate, and (4) morphologic changes in small blood vessels. Above all there seems to be present a defect in the regulation of insulin secretion, such that the normal stimulus (glucose) elicits at first greater amounts of insulin than needed [*1*]. In time, changes in the beta cell are accompanied by sluggish release of hormone, followed in many instances by cell degeneration. Thus the phases of reactive hypoglycemia, decreased tolerance and overt hyperglycemia succeed each other.

The period between birth (more correctly, conception) and the appearance of decreased glucose tolerance is now termed the "prediabetic" state. It might be more advantageous and proper in the light of present concepts to consider it as phase 1 of diabetes. Since hyperglycemia is not as yet present, this phase of the disease might be called *diabetes pre-mellitus*.

The life history of the diabetic subject would then be divided into three phases: (1) diabetes pre-mellitus, (2) diabetes mellitus, and (3) phase 1 or 2 with neuroangiopathy. Classification for its own sake is, of course, a sign of pedantry, but if its object is to draw attention to natural

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divisions of an area of investigations, and to gaps in our understanding, it may be forgiven and may even prove useful.

The biochemical aspects of phase 1 are almost completely unknown. There are no hints or analogies, nor any serendipitous findings, concerning the nature of the genetic abnormality or of one of its more immediate chemical consequences. Many experimental conditions are known which will so affect the beta cells that, after a period of irregular hypersecretion, irreversible damage is incurred, leading to insulin deficiency. Such situations are the exhibition of large amounts of certain anterior pituitary factors, especially the growth fraction in some species [*8*]; the employment of large doses of the 11-17-hydroxyadrenocorticoids in other species [*9*]; and alloxan and related substances. No significant evidence is as yet available for any parallelism between these diabetic states and the total human disease, except for the metabolic aspects of phase 2. For the study of the *prediabetic* phase we are perforce confined to the ultra refinements of tests of carbohydrate tolerance [*1,2*] since we do not know where else in the metabolism of the body to look for deviations. It is to be expected that within the next year or so we will know whether in phase 1 the diabetic subject has an abnormally high level of pituitary growth hormone. An immunologic assay technic has become available recently and the first preliminary data have been published [*10*].

Studies which promise to provide some insight into phase 1 are those related to insulin synthesis: its storage and release, the form of binding during transit in plasma, and the rate and mode of its breakdown.

The biochemical aspects of phase 2, the hyperglycemic expression of the disorder, have been reviewed often and in extenso. I should like here to draw the attention of the reader to newer and prospective developments and to relate them to the over-all picture of the regulation of intermediary metabolism.

The glucose-transfer theory of insulin action [*11–14*] has been widely confirmed and accepted during the past ten years [*15–18*]. (See however the opinions of Chain [*19*].) It has served to revive and intensify interest in the mode of transfer of sugars and other metabolites across membranes and other cell barriers.

With the possible exception of the hepatic parenchymal cell [*20*], the mammalian tissues seem to possess at or in the plasma membrane a system for the regulated transfer of certain monosaccharides. While the intimate mechanism of the translocation remains unknown, it is almost certainly not a phosphorylative process. It precedes the action of hexokinase and can operate on sugars which are not further metabolized by the cell [*14,16*]. It serves to equalize the concentrations inside and out of the cell and thus, by definition, is not an "active" transport system. Temperature dependence is small or absent [*21,22*]. It proceeds therefore with little expenditure of energy. Once the sugar (e.g., glucose) has entered the cell it is subject to further manipulation primarily by its transformation to glucose-6-phosphate.

In some tissues, notably the intestinal epithelium and probably the kidney tubules, there is, in addition, a system which seems able to "accumulate" glucose for release into the blood stream [*23*]. Thus intestinal absorption and renal tubular reabsorption become "active," up-hill processes by virtue of the "accumulator" process.

We can distinguish several types of a fundamental process by which the cell regulates the first step in sugar utilization, namely, transmembrane transport. The basic form seems to be exemplified by the erythrocyte. In this case the sugar molecules are handled by a specific system which facilitates their entry into the cell interior until their concentration in the cell water approaches that of the extracellular fluid [*24,25*]. The mechanisms involved are unknown; the kinetics of the process point to a saturable component or condition of the membrane, be it a "carrier," a specially shaped "hole," or a vesicle. Phlorhizin in low concentration inhibits this transfer process. Insulin seems not to affect the rate of this basic system. The concentration of intracellular glucose under these conditions depends upon the differential rates of the transfer system and that of the hexokinase system which serves as the first trap directing the flow of glucose inward.

A more complex picture is offered by such cells as those of the intestinal epithelium (and probably the renal tubule). Here the basic transfer mechanism seems to be very similar to that of the erythrocyte, but the glucose which has found its way into the cell is subject to two influences. A portion is phosphorylated by the hexokinase system, the bulk seems to be segregated by a "pumping" system. The internal concentration rises above that of the exterior medium. Probably a diffusion process operating towards the serosal

surface of the cell allows the free glucose to run "downhill" into the blood. As in the red cell, the primary transfer process is inhibited by phlorhizin; the "accumulator" mechanism seems to be linked to the "sodium pump." Na^+ on the luminal side of the cell seems to be necessary for "active" transport, i.e., concentration against the gradient [*23*]. Again insulin exerts no influence on the rates of sugar absorption or renal tubular reabsorption. As a matter of fact, intestinal absorption of sugar is markedly increased in the alloxan-diabetic rat [*26*]. In my opinion, this phenomenon may be due to the increased metabolic rate (thyroid ?) noted early in experimental pancreatic diabetes.

Muscle (striated and cardiac), fat cells and fibroblasts seem to possess still another modification of the system for glucose intake. When such tissues as the rat diaphragm are removed and handled with the least possible injury [*27*], sugar uptake is very slow and the concentration of intracellular sugar approaches zero. The addition of insulin to the medium immediately stimulates transfer. The intracellular concentration of glucose does not rise, because of the activity of hexokinase. When glucose phosphorylation is decreased (at low temperatures, by adrenaline, etc.), the internal concentration of the free sugar rises and begins to approach that of the external medium. There is never an "accumulation" of free sugar internally. It is as if muscle and fat cells have acquired a closing or inhibiting device which ordinarily screens the membrane transfer system and does not allow access of sugar to it. Insulin is the hormonal substance which "opens" the door in front of the transfer system. Muscular work (by humoral means possibly) has an effect identical with that of insulin on this system [*28*]. So do anerobiosis and drugs which also uncouple oxidative phosphorylation (dinitrophenol, salicylate, etc.) [*29*]. Such "opening" actions are reversible and are therefore not due to non-specific damage of the cell membrane.

It will be noted that, in the context of sugar transfer as well as in the discussions to follow, cell ultrastructure becomes of fundamental importance to our understanding of biochemical events. In regard to the cell membrane, only a beginning has been made in elucidating its molecular architecture. The membrane proper seems to be a "sandwich" consisting of a lipid filling held together by two layers of protein material. The total thickness is about 75 Å. On its external surface the cell membrane is contiguous with a layer of ground substance material, the basement membrane, containing for the most part protein-polysaccharide complexes [*30*]. The cell surface is most probably not homogeneous at the molecular level but may be studded with specialized patches able to perform specific biochemical functions. Our present lack of insight into this aspect of ultrastructure prevents us from being able to define sugar transport and the modifying effect of insulin in more precise terms.

The first metabolic reaction of intracellular glucose is its transformation to glucose-6-phosphate by ATP, a reaction catalyzed by hexokinase. The term "hexokinase" refers to a family of enzymes differing in ranges of substrate specificity from tissue to tissue, but all capable of phosphorylating glucose. In the diabetic animal the activity of these liver and muscle enzymes is depressed but this decreased activity is not immediately reversible by insulin *in vitro* [*31*]. The phenomenon is not due to a diminished quantity of enzyme since the activity in muscle is brought back to normal values by a short period of anerobiosis. The evidence at this point makes it probable that this inhibition of the rate of sugar phosphorylation is due to the action of pituitary and adrenal cortical factors on hexokinase or phosphohexokinase [*31*]. This phenomenon is consistent with the suggestion made some years ago [*14*] that lack of insulin may lead to heightened anterior pituitary activity (*vide seq.*).

We shall now consider one of the pathways by which glucose-6-phosphate is metabolized, namely, its conversion to glycogen. It is first transformed to glucose-1-phosphate by the enzyme phosphoglucomutase. This ester reacts with uridine triphosphate to form uridine diphosphoglucose and inorganic pyrophosphate. The uridine diphosphoglucose under the influence of the enzyme, glycogen synthetase, transfers its glucosyl group to carbon 1 of the terminal glucose of the outer branch of a glycogen molecule, thus lengthening it. A second enzyme, the branching factor, is able to attach the reducing end (carbon 1) of a straight chain to carbon 6 along the polysaccharide, giving rise to branching. Thus glycogen grows by accretion. It exhibits great heterogeneity in "molecular" size [*32*]. The glycogen aggregates are structurally contiguous to the glycogen synthetase system [*33*].

Breakdown of glycogen is achieved by the joint

action of two enzymes, namely, phosphorylase and amylo-1:6-glycosidase (or debranching enzyme). Phosphorylase activates the cleavage of the 1:4 glycosidic bond at the end of a branch, forming glucose-1-phosphate. When a branch point is reached (a 1:6 link), the debranching factor leads to its hydrolytic cleavage, liberating free glucose, and making another polysaccharide branch accessible to phosphorylase. Phosphorylase can exist in an inactive form. Reactivation is facilitated by activity and adrenaline in muscle, and by adrenaline and glucagon in liver [*32,34*]. In the absence of insulin the level of glycogen decreases sharply in liver, moderately in striated muscle, and remains unchanged in such organs as the brain. However, the amount of glycogen is increased in the kidney tubule and rises sharply in heart muscle during the diabetic state. While the increased renal tubular glycogen may be a reflection of the large amounts of sugar presented to these cells in diabetes, the abnormally high cardiac glycogen seems to be related to increased anterior pituitary activity [*35*]. It is found in fasting and in phlorhizin diabetes (despite hypoglycemia) and does not occur under those conditions in the hypophysectomized animal.

It has been suggested recently that insulin activates the glycogen synthetase system, which would explain why, under some circumstances in muscle, insulin seems to favor this pathway of glucose disposal [*36*]. Such directional effects of insulin are not seen with fat cells [*37*] or with muscle when other conditions do not favor glycogen synthesis [*38*]. It may well be that the "molecular patch" on the membrane forming the glucose transport system is structurally closely related to the glycogen-forming and storage portions of the muscle cell, permitting better initial flow of glucose to that pathway.

It should be noted that the enzymatic separateness of glycogen synthesis from that of breakdown is in harmony with similar events in relation to proteins and triglycerides. Such enzymatic cycles offer an opportunity for directional control and it is at such points that one may expect that the hormones exert their effects (direct or indirect) on metabolic regulation.

In some organs, notably the liver, there is enzymatic provision for hydrolytic cleavage of glucose-6-phosphate, liberating free glucose. The phosphatase involved is specific for this sugar ester, and its activity is subject to wide fluctuations, governed by changes in nutritional and/or hormonal conditions. For example, fasting, lack of insulin and the administration of glucocorticoids increase sharply the activity of glucose-6-phosphatase whereas carbohydrate feeding, insulin and adrenalectomy lower it. The available data point to the tentative conclusion that these effects follow increases or decreases in the rate of hepatic gluconeogenesis and are not the result of direct hormonal action. Again as in the glycogen cycle, the opportunity for directional control is due to the separateness of the systems for the formation and breakdown of glucose-6-phosphate [*39*].

Let us now consider another of the pathways for the transformation of glucose-6-phosphate, namely, the pentose shunt. For the limited purposes of this discussion it will suffice to consider the first reaction of this cycle, the reaction by which glucose-6-phosphate is oxidized to 6-phosphogluconic acid. The enzyme involved is glucose-6-phosphate dehydrogenase and the coenzyme is triphosphopyridine nucleotide (TPN). This coenzyme is reduced during the reaction, and the resulting $TPN.H_2$ is used to provide the hydrogen for certain reductive syntheses, notably the formation of the hydrogen-rich fatty acids from oxygen-rich acetate. Lipogenesis from carbohydrate therefore requires the simultaneous activity of the shunt system [*40,41*]. In accord with this concept is the structural association of the shunt enzymes with the nonmitrochondrial system for fatty acid synthesis in one subcellular "particle." The activity level of glucose-6-phosphate dehydrogenase is markedly affected by nutritional conditions [*42,43*]. It varies directly with carbohydrate load (especially in the fat tissues) and depends upon the presence of insulin to allow for rapid entry of glucose into the cells. Under normal conditions an ingested carbohydrate load is disposed of principally by its more or less temporary conversion to fat. One of the important secondary metabolic defects in the total diabetic subject is the interference with reductive synthesis of fat because of the inadequate supply of $TPN.H_2$ produced at or near the site of lipogenesis.

Recently some unwelcome clouds of experimental facts are seen to invade this clear blue concept. There is evidence, for example, to suggest that the reactions of fatty acid synthesis in some manner activate the shunt system: a reversal of the gears. During the past two years a different mode of lipogenesis from acetate has been uncovered which can use $DPN.H_2$, al-

though the rates *are* faster with $TPN.H_2$ [*44*]. Discussion of this aspect of metabolism will be resumed subsequently.

The various steps in the glycolytic breakdown of glucose-6-phosphate to acetate have been known for years. Attention may be drawn, however, to two reactions important in metabolic control. The enzyme, phosphofructokinase, transforms fructose-6-phosphate to fructose-1,6-diphosphate. The reverse reaction is not catalyzed by the same enzyme system. There exists a specific fructose diphosphatase which cleaves the phosphate attached to carbon 1. This enzyme is governed in activity by the degree of gluconeogenesis, in the same manner as is glucose-6-phosphatase [*45*]. Fructose diphosphate is split to two trioses, namely, glyceraldehyde phosphate and dihydroxyacetone phosphate. From the latter, glycerol phosphate is formed. This compound is important, since by its combination with intracellular free fatty acids it forms triglycerides, which constitute the neutral form of fat stored by the cell [*46*].

The intertwining of the intermediary metabolic pathways for fat and carbohydrate is evident. Glucose degraded via the glycolytic pathway provides the necessary building block, acetate (as the activated form, acetylCoA). The excess oxygen atoms of the carboxyl groups of the acetate are removed by $TPNH_2$, generated from the oxidation of glucose-6-phosphate. When the long-chain fatty acids are formed they are deposited as triglyceride by means of the glycerolphosphate derived from products of glycolysis. In this intertwined but ordered network insulin favors fat synthesis by providing the original substrate, namely, glucose. Adrenaline, on the other hand, facilitates triglyceride breakdown to free fatty acids and free glycerol [*46*]. An increasing free fatty acid concentration in the cell seems to direct carbohydrate breakdown predominantly along the glycolytic pathway, interfering somehow with $TPNH_2$ production by the shunt system. This lipolytic action of adrenaline seems to be aided by the presence of thyroxine and of the glucocorticoids.

The cell may have more than one pathway for fatty acid synthesis. Until recently, lipogenesis was thought to proceed essentially by the repetitive attachment of acetyl CoA molecules, followed by reduction at the alpha carbon of the growing chain, until palmityl-CoA was formed. Intensive work in this field during the past few years favors the concept that acetyl CoA is acted upon by a catalytic system (aided by biotin) which leads to the addition of carbon dioxide to the molecule, thus forming malonyl CoA. In the presence of another enzymatic system, 7 molecules of malonyl CoA and 1 molecule of acetyl CoA condense to form 1 molecule of palmitoyl CoA, and liberate 7 molecules of carbon dioxide and 7 of free CoA. $TPN.H_2$ is the more effective of the pyridine nucleotides for reduction [*44*].

It has already been stated that fasting and/or insulin lack interfere with the normal degree of fat synthesis. A potent factor of unknown chemical constitution is obtainable from livers of fed rats which will greatly stimulate lipogenesis when added to enzyme preparations made from liver tissue of fasted rats. This factor has been termed lipogenin and is chemically not related to $TPN.H_2$, insulin or other known catalytic materials [*47*].

The detailed relationships of insulin and of the carbohydrate transformations to protein and amino acid metabolism are not as well known as those in regard to fats. It seems to be increasingly evident that the effects of insulin are involved in amino acid incorporation into protein, both directly and via carbohydrate effects. Insulin increases the rate of transmembrane penetration of the non-utilizable 2-aminoisobutyric acid, but does not affect the rate of cellular accumulation of the normal L-amino acids [*48*]. However, L-amino acid incorporation into protein is hastened by insulin, via its effect on glucose in fat tissue [*49*], and by some other, more direct effect in muscle and liver [*50*]. Insulin seems to be necessary for exhibition of the protein synthesizing effect of pituitary growth hormone, even though the pure "growth" effect of the latter cannot be imitated by insulin [*51*]. The increased urea formation seen in the subject with severe and total diabetes cannot be ascribed solely to the effect of insulin loss because of the well known depression of excessive protein breakdown which occurs in the diabetic animal following hypophysectomy and/or adrenalectomy. The manner by which insulin may be involved in the reaction steps of protein biosynthesis remains totally unexplored.

Having discussed the derangements found (in diabetes mellitus) in tissues such as muscle, fat cells and the heart we turn now to examine the possible causes of the metabolic deviations in the liver of an organism deprived of insulin. Descriptively, such a liver shows increased glycogenolysis and gluconeogenesis, low levels

of glycogen, a rise in the concentration of triglycerides, increased formation of beta-keto-acids and of urea. Some of these phenomena are clearly secondary to the events going on in fat tissues and muscle. In the presence of inadequate glucose intake into these peripheral tissues, the net breakdown of triglycerides to free fatty acids is not restrained by their *in situ* re-esterification. A similar (although yet unknown) mechanism may operate to promote net liberation of amino acids. The influx of amino acids into the liver leads to their transformation into glucose-6-phosphate and keto acids. In some way this stimulates activity of the specific glucose-6-phosphatase, leading to increased glucose output. The increased phosphatase activity tends to favor breakdown of stored glycogen as well. The fatty acids are in part oxidized completely, or esterified to triglycerides, and a major portion is transformed to beta keto acids. The hormonal regulation of these hepatic processes is still far from clear. Hypophysectomy and adrenalectomy effectively restrain ketosis, increased gluconeogenesis and increased hepatic sugar output even in the complete absence of insulin. Is this the result of cessation of increased lipid and protein mobilization alone?; or do pituitary and adrenal hormones affect rates of reaction in the liver as well? Glucocorticoids given to normal animals increase gluconeogenesis but this is accompanied by an increase in hepatic glycogen. Is this due to liberation of additional insulin by the adrenal steroids?

The plasma membrane of the hepatic parenchymal cell seems freely permeable to sugars of many configurations [*52*]. The action of insulin on glucose transfer so clearly evident in the extrahepatic tissues is not discernible in the liver, although it is possible to imagine that a portion of the hepatic cell is partitioned off by a membrane on which insulin would exert its usual effect.

Miles of typewriter ribbon have been worn out during the last few years in the controversy as to whether (1) insulin has any action on the liver cell and (2), if it does, is it direct or via its extrahepatic effect [*20,53,54*]. To me, as of this moment, the status of this debate looks as follows:

The examination of hepatic sugar output by the measurement of C^{14}-glucose specific activity under the influence of insulin has been interpreted as showing [*55*] or not showing [*56*] a significant inhibition of hepatic output by this hormone. However, the evidence is in favor of the inhibition by insulin of increased hepatic delivery of sugar when this comes from increased rates of glycogen breakdown, caused, for example, by reflexly secreted adrenalin, acute phorhizin action or glucagon administration [*57*].

Technics which measure hepatic sugar output by estimating blood flow and arteriovenous differences have also yielded contradictory results. Strikingly positive results (inhibition of output and promotion of net uptake) have been obtained by the use of animals with Eck fistula [*58*], negative results when the portal circulation was not deviated [*59*]. No resolution of this contradiction is yet possible.

The use of the perfused liver as a tool has again yielded mixed findings. Positive data indicating direct effects of insulin have been appearing in recent years, to counter the host of negative experiments reported previously. Lipogenesis, amino acid incorporation and potassium entry have been stimulated when insulin was added to the perfusate [*60–62*].

The data cited do not as yet permit surmises about the modes of insulin action on the liver, but they are sufficient to incline one to abandon the completely negative viewpoint expressed before.

Another indirect means by which insulin may exert important normalizing effects on liver and extrahepatic metabolism in the diabetic subject may be its direct influence on the anterior pituitary [*14*]. If this gland is indeed insulin-sensitive in regard to sugar uptake, it may well regulate its output of certain hormones, depending upon the rate of carbohydrate turnover. In the untreated, completely depancreatized dog, the oxygen consumption of both the liver and the extrahepatic tissues is increased (increased thyroid stimulating hormone?). There is an increase in cardiac glycogen (growth hormone) in diabetic animals [*35*] and muscle hexokinase activity is decreased (growth hormone). How much of the increase in lipid and protein mobilization is due to the lack of re-synthesis following insulin withdrawal and how much is the result of increased breakdown due to pituitary and adrenal cortical activity? Insulin may inhibit gluconeogenetic phenomena in part by restraining the secretory activity of the anterior pituitary.

The biochemical aspects of diabetic angiopathy and neuropathy remain almost wholly unexplored. It is probable that the periodic

acid-Schiff positive material seen in the kidney of subjects with Kimmelstiel-Wilson disease may consist of glycoprotein complexes but its genesis and the underlying cell derangements are unknown. The causative relation of these changes to the disturbances in intermediary metabolism herein discussed is doubtful. Experimental destruction of the beta cells does not lead to the typical angiopathy of diabetes despite lack of adequate control of the "diabetic metabolic" derangements.

In attempting to cover this vast and fascinating subject, the biochemistry of diabetes, one perforce has to be selective in his choice of reported work. The selection is determined by available space and time, and also by a viewpoint which inevitably leads to changes in emphasis of one or another direction of research activity in a field. Without using the cliché that "more work is needed in this area," it should be evident that the uncharted areas are far more extensive than the known waters, closer to the shore.

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Ketogenesis and Hyperketonemia*

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ALTHOUGH the storage capacity of the body for fat is exceedingly flexible, the ability of the human organism to maintain a carbohydrate reserve is limited to less than 400 gm. [*1*]. Thus in the average well nourished person, 90,000 calories may be stored as fat and only 1,500 as carbohydrate [*2*].

At the same time, approximately 120 gm. of glucose per day is required to maintain the central nervous system, and an additional unspecified quantity of carbohydrate may be needed to support important functions in other parts of the body [*3*]. For example, muscle glycogen must be available to provide energy during periods when the demand for energy expenditure exceeds the ability of the circulation to provide oxygen. Small amounts of carbohydrate may be needed in the synthesis of certain enzymes, nucleic acids, glycoproteins and to replace 4-carbon intermediates that may be lost from the tricarboxylic acid (TCA) cycle by attrition.

From these facts it is clear that when carbohydrate reserves become depleted the body must be able to draw promptly upon its fat stores to maintain energy balance and to conserve carbohydrate for essential purposes. At the same time, mechanisms must be available for rapid manufacture of carbohydrate from non-carbohydrate precursors since the supply of glycogen stored in the liver can at best sustain the brain (which utilizes only carbohydrate) for about one day.

It is helpful to consider the phenomena of ketogenesis and ketosis in the context of this potentially critical need for carbohydrate conservation and fat utilization.

NORMAL MECHANISM OF KETOGENESIS

Ketogenesis is a normal function of the liver and the so-called ketone bodies are normal substrates for metabolism [*4*]. Indeed, the ketones probably constitute an important transport form of energy derived from fat that the liver provides for various working cells. For example, it has been postulated that the ketosis which takes place *after* but not during exercise (Courtice-Douglas effect) results from an accelerated production of ketone bodies by the liver [*5*]. During exercise there is an increased oxidation of these substances by the working muscles; hence, hyperketonemia does not occur. After exercise stops, extrahepatic utilization of ketone bodies decreases at once but hepatic overproduction continues for a time.

Since acetone and β-hydroxybutyric acid are derived from acetoacetic acid, as indicated in Figure 1, this discussion will be concerned primarily with factors that influence synthesis of acetoacetic acid. (It has been reported that

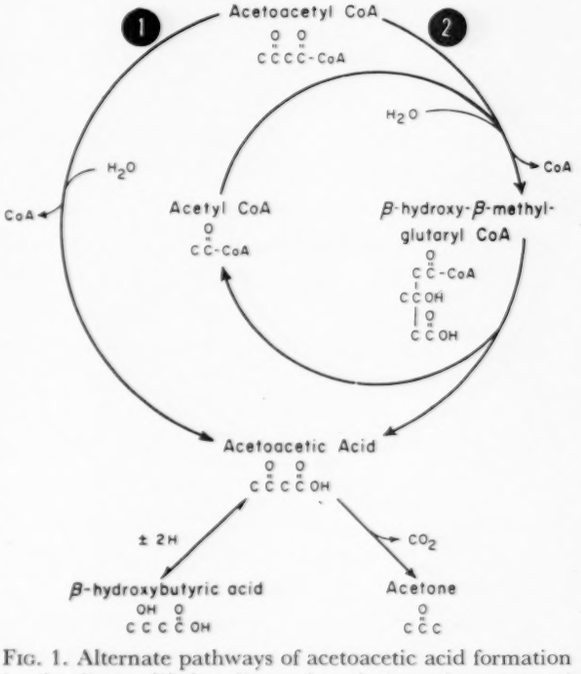


FIG. 1. Alternate pathways of acetoacetic acid formation in the liver: (1) by direct deacylation of acetoacetyl coenzyme A, (2) by cleavage of the intermediate six-carbon compound, β-hydroxy-β-methyl glutaryl coenzyme A (HMG-CoA cycle).

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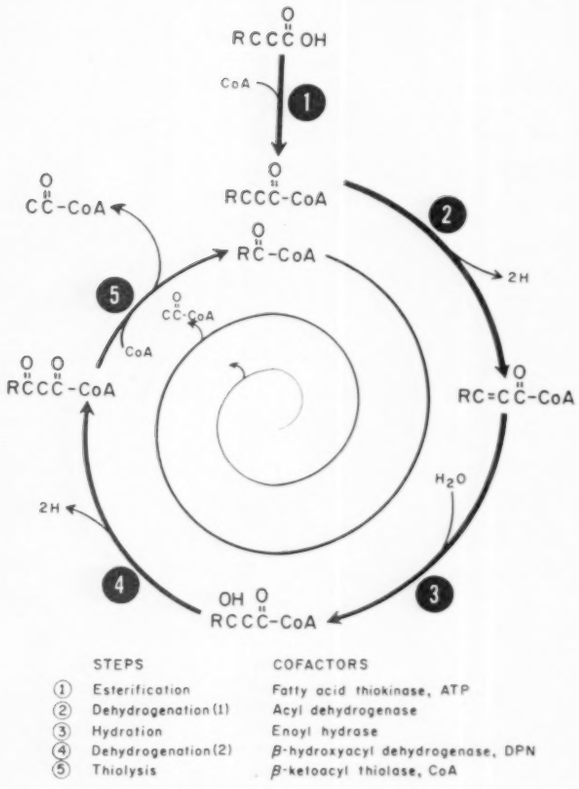


FIG. 2. Steps in the oxidative generation of acetyl coenzyme A from a long-chain fatty acid.

acetone, which is produced by decarboxylation of acetoacetic acid, can form pyruvic acid [*6*]. If this pathway proves to be appreciable, ketogenesis may represent one route, albeit inefficient, whereby fatty acid carbon can be used for net synthesis of carbohydrate.)

In the liver, ketogenesis is inseparable from the process of fatty acid degradation and oxidation. The basic sequence which by repetition accounts for fatty acid breakdown is represented by the following summary equation.

$$RCH_2CH_2COOH + 2H_2O \xrightarrow{-4H} RCOOH + CH_3COOH$$

The component steps needed to accomplish the cleavage of one acetate fragment from its parent acid are indicated in Figure 2. Each degradative sequence produces one molecule of acetyl coenzyme A (acetyl CoA) and a shortened fatty acid which is primed by esterification with a second molecule of coenzyme A (step 5) for removal of another 2-carbon unit [*7*].

The further fate of acetyl CoA is determined by metabolic circumstances. As shown in Figure 3, alternate routes include condensation with oxaloacetate to form citrate, which is further oxidized in the TCA cycle. Two molecules of

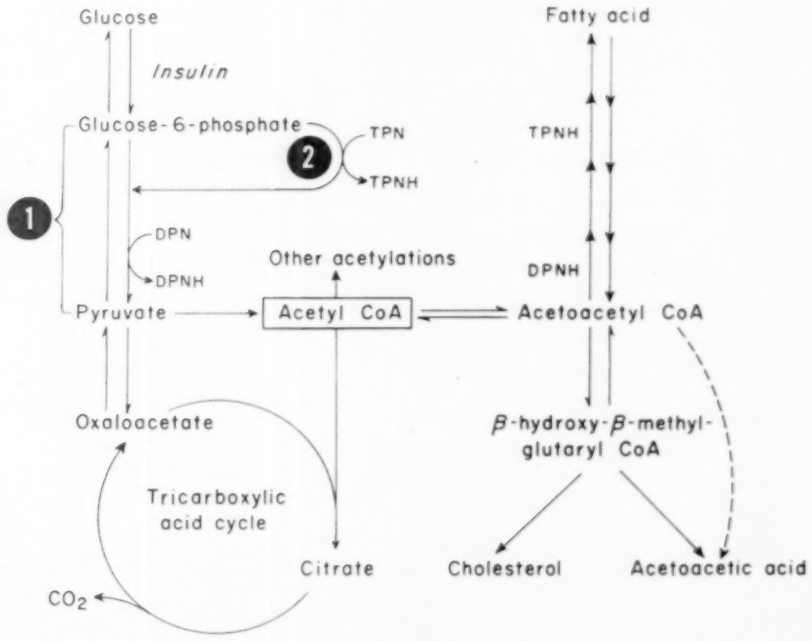


FIG. 3. Certain interrelationships between glucose and fatty acid metabolism in the normal liver. Embden-Meyerhof (1) and pentose phosphate (2) pathways generate cofactors that may be rate-limiting in lipogenesis.

acetyl CoA can also condense to form acetoacetyl CoA which, in its turn, can participate in lipogenesis, ketone body formation or cholesterol biosynthesis.

According to Green and Gibson [7], in liver mitochondria, fatty acid oxidation proceeds partly to completion (to CO_2 and H_2O), while the rest goes no further than the stage of acetoacetate. There is an enzyme in liver (deacylase) that catalyzes the deacylation of acetoacetyl CoA to acetoacetate and CoA. This particular reaction is irreversible. At the same time the liver seems to be deficient in the enzyme (acetoacetate thiokinase) that catalyzes the acylation of CoA by acetoacetate. Accordingly, acetoacetate is formed in the liver while in other tissues it tends to remain bound to CoA.

Lynen and his associates [8] have suggested that deacylation of acetoacetyl CoA does not take place directly but involves two steps: (1) condensation of acetoacetyl CoA with acetyl CoA to form β-hydroxy-β-methyl glutaryl CoA (HMG-CoA), and (2) cleavage of this 6-carbon substance to form acetoacetate and acetyl CoA. (Fig. 1.) On the other hand, Drummond and Stern [9] have identified an enzyme in beef liver that can catalyze direct hydrolysis of acetoacetyl CoA. In their enzyme preparation, complete inhibition of HMG-CoA cleavage enzyme with iodoacetamide did not decrease the rate at which acetoacetate was formed from acetoacetyl CoA. Such experiments suggest that formation of free acetoacetate need not occur via the so-called "HMG-CoA cycle" but may take place by direct deacylation [10].

In view of the elevated serum cholesterol levels reported for certain poorly controlled diabetic patients [11], it is noteworthy that HMG-CoA is the immediate precursor of mevalonic acid, a compound now recognized to be crucial in cholesterol biosynthesis.

Thus, acetoacetate can arise from the synthesis of acetoacetyl CoA by the condensation of two 2-carbon fragments or, possibly, as a direct product of fatty acid degradation in the liver. Crandall and Gurin [12], and Geyer et al. [13] have demonstrated in *in vitro* studies that the terminal CH_3CO— unit of a fatty acid chain is more likely to be found in acetoacetate than the —CH_2CO— units. Whether the CH_3CO— unit enters the TCA cycle less readily than the —CH_2CO— unit, serving instead as an acetylating unit in the formation of acetoacetate, or whether, in the liver, some proportion of fatty acid degradation does not progress beyond acetoacetate formation is not entirely clear. Perhaps both mechanisms are operative.

It is interesting that under *in vitro* conditions more acetoacetic acid is formed by liver mitochondria from shorter chained fatty acids (C_6-C_{10}) than from the long chain fatty acids (C_{16}-C_{18}) [14]. Under the same conditions the longer chained fatty acids form more CO_2. In one study on twenty-four healthy subjects, Schön et al. [15,16] administered 100 gm. of a medium chain glyceride (MCG) containing equal proportions of C_8, C_{10} and C_{12} fatty acids. Blood ketones increased significantly after the administration of the MCG preparation, but there was little or no rise after the ingestion of an isocaloric quantity of margarine.

Thus in the liver, acetoacetate formation appears to occur normally in association with fatty acid catabolism. The extent to which ketones are formed as direct terminal products of fatty acid degradation still is not evident. If ketone manufacture by such a mechanism were appreciable, it would be possible to relate ketogenesis more directly to the rate of fatty acid catabolism, and perhaps to the rate of delivery of free fatty acids (FFA) to the liver. On the other hand, it is known that acetoacetate arises from condensation of acetate fragments. If this latter mechanism of ketogenesis is predominant (and a great deal of evidence suggests that it is), the rate of ketone production will depend to a greater extent upon the ability of the liver to dispose of acetyl CoA by routes other than ketogenesis. (Fig. 3.)

In any event, the fact that the liver enzymes are oriented toward ketone production and not utilization, and that the blood always contains significant quantities of ketones [17] argues strongly for the concept of ketogenesis as an important mechanism for the provision of transportable energy to extrahepatic tissues.

HYPERKETONEMIA

Hyperketonemia is most often found as a consequence of starvation, excessive fat intake, carbohydrate privation or diabetes mellitus. Hyperketonemia also occurs in certain lactating ruminants [18], animals with phlorizin-induced diabetes [19] and fasting pregnant animals [20]. All of these conditions probably have in common some degree of carbohydrate deficiency, increased fat utilization and increased gluconeogenesis.

It cannot be assumed that the ketosis induced by fasting is metabolically the same as that occurring in diabetes. There is growing evidence that the pattern of enzyme activity in liver slices from starved, ketotic rats is different from that in liver preparations from alloxan diabetic rats even though the severity of the ketosis may be the same in both instances. Wieland and associates [*21*] have reported that the activity of the enzyme concerned with acetoacetate synthesis does not increase during starvation but increases markedly in the diabetic liver. In livers from starved animals there is a striking decrease in activity of HMG-CoA reductase, and in cholesterol synthesis; in diabetic livers similar decreases do not occur.

Also, there have been reports that the ketosis induced in animals by a butyrate-rich diet is ameliorated by the administration of oxaloacetate while the ketosis associated with alloxan diabetes remains unresponsive to treatment with oxaloacetate and other TCA cycle intermediates [*22–24*]. Whether such metabolic discrepancies represent differences in kind or in degree remains to be determined.

In one study on three diabetic patients in the precomatose stage of ketoacidosis hepatic output of ketone bodies ranged from 0.12 to 4 mg. per minute per kilogram body weight [*25*]. By contrast, the average rate of ketone production in sixteen non-diabetic men in the postabsorptive state was approximately 0.05 mg. per minute per kg. [*26*].

In diabetic coma the blood ketone concentration may range from 100 to 200 mg. per 100 ml.; in normal fasting subjects ketone levels average 5 mg. per 100 ml. [*17*]. When ketosis is induced in normal subjects by nutritional means, ketone levels may rise to 25 mg. per 100 ml. [*17*].

There is abundant evidence that extrahepatic cells can readily oxidize ketone bodies. The mechanism presumably involves acylation of CoA by the ketone with subsequent formation of acetyl CoA which, in turn, enters the TCA cycle. Although earlier infusion studies suggested that diabetic organisms are able to dispose of ketones with a rapidity equal to that of non-diabetic control animals [*27*], a recent report indicates that acetoacetate uptake by diaphragm and skeletal muscle of diabetic rats *in vitro* may be reduced [*28*]. Thus, in diabetic states, the possible role of decreased ketone utilization by extrahepatic tissues in promoting hyperketonemia requires re-evaluation.

Metabolic Changes in Diabetes Mellitus. To approach the problem of the pathogenesis of ketosis consideration must be given to certain metabolic changes that occur in starvation and diabetes mellitus.

Normally, diurnal variations occur in utilization of carbohydrate and fat in relation to meals. By three or four hours after a meal containing carbohydrate, alimentary hyperglycemia has given way to "fasting" levels, utilization of blood glucose has decreased and a reciprocal rise in plasma free fatty acids and ketones has occurred. Following ingestion of a carbohydrate-rich meal, there is an increase in glucose utilization. Release of free fatty acids from the fat depots is reduced [*29*] and blood ketones decrease [*30*]. The diurnal pattern of changes of free fatty acids in relation to peripheral glucose uptake in a group of normal subjects is shown in Figure 4.

During the phase of alimentary hyperglycemia and enhanced glucose utilization certain biochemical events take place in hepatic cells that merit comment. When hyperglycemia occurs, glucose enters liver cells and, in the presence of insulin, is phosphorylated to glucose 6-phosphate. (Fig. 3.) From this point it can be degraded to acetyl CoA by either of two routes: (1) The Embden-Meyerhof (EM) pathway via fructose and triose to pyruvate and then acetyl CoA, or (2) the pentose phosphate pathway via a 5- and then a 7-carbon compound to fructose and triose, and thence along a route shared by the EM pathway to acetyl CoA.

During alimentary hyperglycemia the supply of carbohydrate may exceed the capacity of the body to use this material for energy or store it as glycogen. At such times lipogenesis becomes very active in both liver and adipose tissue and the acetyl CoA fragments that are not used for oxidative purposes participate in fatty acid synthesis [*31*].

For the purposes of this discussion, several details need to be emphasized: (1) During the course of glucose catabolism in the pentose phosphate "shunt," the reduced form of the coenzyme triphosphopyridine nucleotide (TPNH) is produced. This cofactor appears to be needed for the manufacture of fatty acids from acetate fragments. Reduced diphosphopyridine nucleotide (DPNH), which is generated during EM glycolysis, also is needed for fatty acid synthesis [*32*]. (2) Before acetyl CoA can enter the TCA cycle and be further oxidized, it must

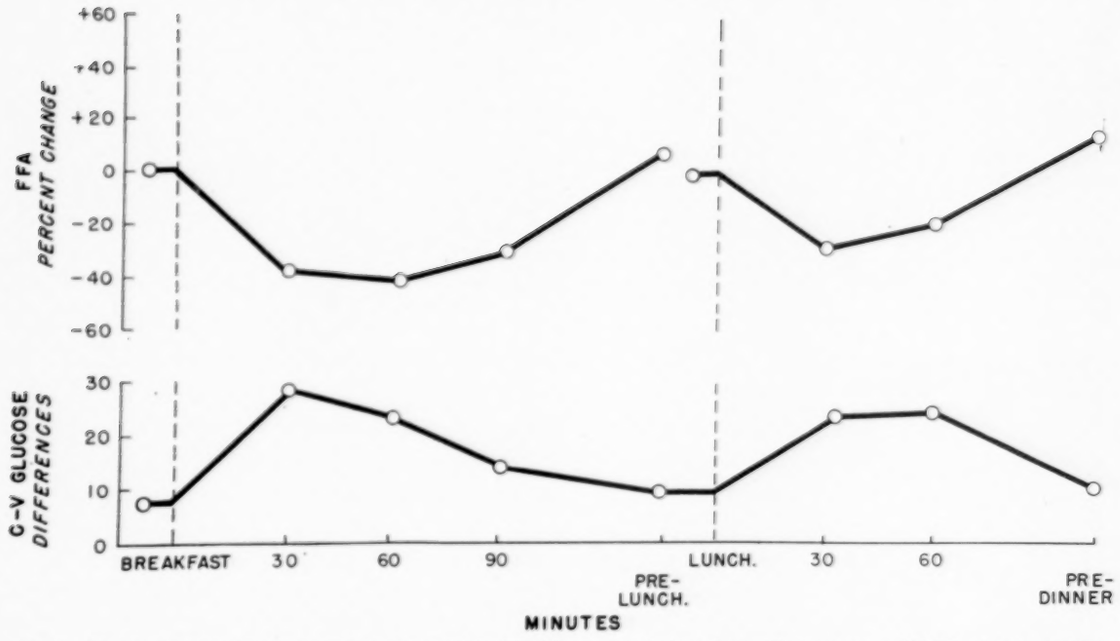


FIG. 4. Mean changes in plasma free fatty acids (FFA) and peripheral capillary-venous (C-V) glucose differences in nine healthy subjects in relation to ingestion of a "normal" breakfast and luncheon [*29*].

condense with oxaloacetate, a compound that under normal metabolic circumstances is derived almost entirely from carbohydrate. (3) The transformation of pyruvate to acetate is unidirectional. Acetyl CoA cannot form pyruvate and hence cannot be used for net glucose synthesis. It is for this reason that, in man, fatty acids cannot be converted to carbohydrate via acetyl CoA, although incorporation of acetate carbon into hexose can occur via the TCA cycle and possibly by other pathways.

In the phase of decreasing carbohydrate utilization, and to a much greater degree in the carbohydrate-deficient person, including the patient with untreated diabetes mellitus, glycolysis via both pathways is reduced. It has been reported that in the liver the proportion of glucose 6-phosphate ordinarily metabolized by the pentose phosphate route can range from 5 to 50 per cent [*33*]. In the liver of the diabetic animal, glucose phosphorylation may be reduced by 75 to 90 per cent and a marked net decrease occurs in the rate at which glucose is degraded along the glycolytic pathways. Thus the supply of TPNH (and possibly DPNH) available for lipogenesis is reduced, and at the same time synthesis of fat from acetyl CoA is suppressed.

Several observers [*32,35*] have emphasized that glycolysis acts as a generating system for the reduced pyridine nucleotides and have suggested that it is through these coenzymes, in particular TPNH, that glucose metabolism may exert a regulatory influence on lipogenesis. This theory still requires adequate confirmation.

In the liver of the diabetic patient or starved subject a large increase in gluconeogenesis from protein occurs. There is an increase in the activity of the enzyme, glucose 6-phosphatase, which promotes release of glucose from the liver [*36*]. At the same time, glucokinase activity, responsible for phosphorylation of incoming glucose, is reduced [*37*]. Thus the major flow of traffic is *up* the EM pathway, starting with amino acid conversion to pyruvate and other glucose precursors. The enzymes that promote glucose synthesis from 3-carbon intermediates also show increased activity [*38*].

In the diabetic liver, then, two major changes in metabolic behavior (among the many that occur) are noted: (1) suppression of fatty acid synthesis from acetyl CoA fragments, and (2) greatly increased gluconeogenesis from appropriate amino acids.

THEORIES OF DIABETIC HYPERKETONEMIA

Of the various theories to account for diabetic hyperketonemia three have received considerable attention and also serve to illustrate the range of explanations that have been offered. They might well be called (1) the fatty acid

plethora theory; (2) the oxaloacetate deficiency theory; and (3) the gluconeogenesis theory.

(1) The fatty acid plethora theory has roots in experiments performed by Embden at the turn of the century [*39,40*]. He and his associates perfused the mammalian liver with even-numbered straight-chain fatty acids with the resulting production of acetoacetic acid and acetone. Other workers have shown that when a normal human liver is loaded with an infusion of octanoate it may increase its ketone production fivefold [*40*]. More recently, Dole [*41*] and others [*42*] have revived this theory in the light of new knowledge about the physiology and metabolism of free fatty acids. It has been demonstrated that in "acute diabetes" there is a marked increase in rate of release of free fatty acids from adipose tissue. Hence the free fatty acid level in diabetic acidosis may rise to three times normal, or more [*42,43*]. It is believed that the free fatty acid fraction, although comprising less than 10 per cent of plasma fatty acid, turns over very rapidly. One estimate of free fatty acid half-life is one to three minutes [*44*]. Bogdonoff and his associates [*45*] have calculated that 25 per cent of the free fatty acid pool turns over each minute when free fatty acid levels are 1,000 μEq. per L. or greater. (Such free fatty acid levels are common in patients with uncontrolled diabetes mellitus.)

As Dole [*41*] has pointed out, in the labile diabetic patient who fails to receive his morning dose of insulin, plasma free fatty acids rise sharply, preceding the appearance of hyperketonemia. After ketosis has occurred, insulin treatment induces a prompt fall in free fatty acids which precedes by a matter of hours disappearance of ketone bodies from the urine. The liver appears to extract circulating free fatty acids roughly in proportion to their concentration in the perfusing blood [*46,47*]; hence, the liver becomes burdened with an excess of fatty acids of which it must dispose.

Two alternatives seem possible: first, the liver could re-esterify the fatty acids into glycerides and, possibly, phospholipids and cholesterol esters and store or release them as moieties of lipoprotein molecules. Since the liver tends to accumulate fat, and the serum concentration of low density lipoprotein rises during episodes of poorly controlled diabetes, this pathway of fatty acid disposal clearly is utilized. However, increasing accumulation of neutral fat in the liver may inhibit triglyceride biosynthesis; moreover, the supply of glycerol needed for glyceride manufacture may be limited in the diabetic liver.

Second, the fatty acids could be degraded to acetyl CoA fragments or, perhaps, directly to acetoacetic acid. Since there are more 2-carbon units produced than can be accommodated in the TCA cycle and since lipogenesis is inhibited in the diabetic state, a major remaining alternative is for the liver to synthesize and release into the circulation a large number of ketone bodies.

Thus, according to the "fatty acid plethora" theory, production of acetoacetate in the liver might well relate fairly directly to the rate at which fatty acids are delivered to and catabolized in this organ.

Recently, the belief that the TCA cycle functions normally during ketosis has been challenged by Wieland [*48*]. He has pointed out that 1.6 times more hydrogen is generated per adenosine triphosphate equivalent when fatty acids are degraded to ketones than in glycolysis. Thus, when an increasing proportion of fatty acids is metabolized within liver cells, as in diabetes mellitus, the potentials of key oxidation-reduction systems are lowered with a resulting suppression of TCA cycle activity.

Wieland has reported results of experiments involving perfusion of rats' livers with caprylate in which the relationship between formation of ketone bodies and oxygen consumption was determined. He and his associates found that if the calculated quantity of oxygen used for ketone formation is subtracted from total respiration, the amount of oxygen used to support terminal (TCA cycle) oxidation decreases as more and more fatty acid is metabolized. Wieland concludes that the velocity of the TCA cycle in the liver is controlled by the rate of fatty acid oxidation and that, in diabetes, increased ketogenesis results from an increased rate of combustion of fatty acids in the liver associated with a concomitant decrease in TCA cycle activity.

(2) The "oxaloacetate deficiency" theory is based on the concept that in diabetes a deficiency of phosphorylated glucose within the liver cell results in a deficiency of oxaloacetate. Thus, during the increased fatty acid catabolism that occurs in the diabetic liver, the numbers of acetyl CoA units that arise exceed the supply of oxaloacetate molecules with which to condense. Hence the 2-carbon fragments derived from fatty acid breakdown accumulate in liver

cells and combine to form acetoacetic acid. Support for this theory is provided by experiments showing that when the tricarboxylic acid cycle in liver slices is blocked by malonate and other agents, acetoacetate is produced in increasing amounts [*49*]. Recknagel and Potter [*50*] have demonstrated in rat liver slices that, in the presence of an excess of ammonium ions, alpha-ketoglutarate (a TCA cycle intermediate) is removed by reductive amination to form glutamic acid. In this way, the cycle is interrupted and, coincidentally, ketone body formation is enhanced.

Baldwin [*51*] has suggested that the rate of depletion of oxaloacetate in the liver may be greater than that in other tissues because of the presence of the hepatic enzyme, oxaloacetate decarboxylase which catalyzes the breakdown of oxaloacetate to pyruvate [*52*]. Thus the liver may have a special need for carbohydrate in order to replenish its supply of oxaloacetate.

It might be imagined that it would be relatively simple to determine whether diabetic hyperketonemia results from an hepatic deficiency of oxaloacetate. Unfortunately, studies designed to answer this question have yielded conflicting results. It has been reported that the level of oxaloacetate in the liver of the alloxan-diabetic rat is normal [*53*]. Moreover, with the exception of the results obtained by Wieland, there does not seem to be any impairment in rate of respiration in liver preparations from diabetic rats [*54*], and diabetic ketosis in alloxan-treated dogs is not responsive to intravenously administered oxaloacetate or citrate [*22*]. However, it is interesting that parenterally administered oxaloacetate does eliminate ketosis induced by a diet rich in butyrate in otherwise normal rats [*22*].

(3) Fifteen years ago Peters [*4*], in a discussion of ketone metabolism, made the following statement: "When the evidence is all summed up it seems to indicate that ketogenesis increases when protein assumes the functions of carbohydrate, including the formation of hepatic glycogen." To support this view, he cited the close parallel between wastage of nitrogen and ketogenesis in persons subjected to experimental starvation. Gamble and associates [*30*] observed a similar correlation between nitrogen deficit and hyperketonemia in persons participating in life-raft survival experiments during World War II. It is also noteworthy that hypophysectomized and adrenalectomized animals are resistant to ketosis [*55,56*], although their hepatic glycogen becomes rapidly depleted in the absence of dietary carbohydrate. Such animals have difficulty in manufacturing new carbohydrate from protein, but they display a reduced rate of fat mobilization as well.

Although there is an association between increased gluconeogenesis from protein and increased ketogenesis, most observers have assumed the underlying cause of both phenomena to be carbohydrate deficiency. However, a recent and more sophisticated version of the gluconeogenesis theory has proposed that ketogenesis is linked to gluconeogenesis by a hydrogen transfer system that permits the hydrogen produced during fatty acid oxidation to be used in the synthesis of glucose from 3-carbon precursors [*57*]. In this regard it is an interesting coincidence that four hydrogen atoms are needed for transformation of two molecules of pyruvate into one molecule of glucose, and the same number of hydrogens is released during the production of one molecule of acetyl CoA from a fatty acid such as palmitate.

It has been suggested that excess formation of acetyl CoA and hence of ketones may be a mechanism whereby hydrogen can be provided for the greatly accelerated gluconeogenesis that occurs in the acute diabetic state. From another standpoint, it is possible to conceive of gluconeogenesis as a system for disposal of the excess hydrogen released during the active fatty acid catabolism that accompanies uncontrolled diabetes [*57*].

The proponents of the gluconeogenesis concept support their position by reference to the ketosis of phlorizin diabetes [*19*] and bovine ketosis associated with lactation [*18*]. In both these conditions excessive gluconeogenesis is required to maintain blood glucose above a hypoglycemic level, and associated with the gluconeogenesis is a severe hyperketonemia.

While the notion of linking gluconeogenesis so intimately to ketogenesis is provocative, more information about the intricacies of hydrogen metabolism in the liver will be needed before the gluconeogenesis theory can be adequately evaluated.

SYNOPTIC DISCUSSION

Although the problem of ketogenesis and ketosis has been briefly discussed in terms of separate theories, it is probable that as additional biochemical information accumulates,

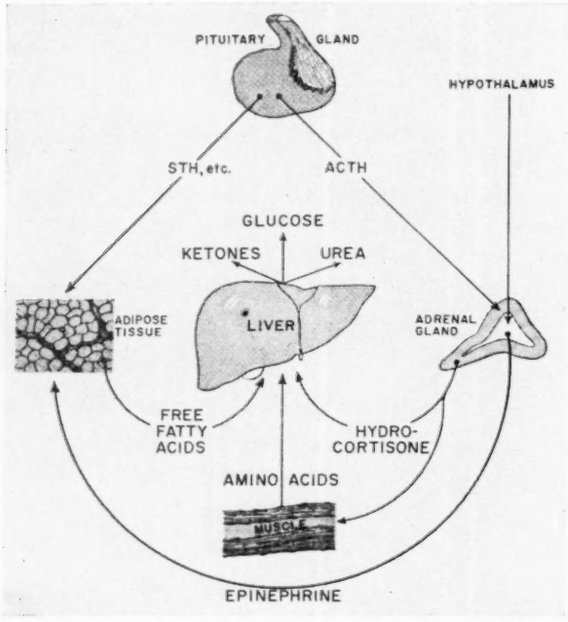


FIG. 5. Postulated endocrine response to cellular deficiency of carbohydrate.

several diverse concepts of ketogenesis will emerge as partial aspects of a more comprehensive picture. In the meantime a tentative synthesis can be made on the basis of facts that are currently available.

There seems to be little doubt that the hyperketonemia of diabetes mellitus is a consequence of manufacture of ketone bodies by the liver at a rate that exceeds the ability of the extrahepatic tissues to remove them. While ketosis is associated with carbohydrate deficiency at the cellular level, the diabetic form probably does not arise from a deficiency of oxaloacetate, a key derivative of carbohydrate. It seems more likely that carbohydrate privation somehow stimulates the mobilization of excessive quantities of free fatty acids from adipose tissue. As a consequence, the liver removes increasing amounts of free fatty acids from the circulation.

Apparently, the ability of the diabetic liver to dispose of the free fatty acid load by glyceride manufacture is limited, and it is well established that lipogenesis from 2-carbon units is suppressed in the diabetic state. The TCA cycle does not increase its activity to accomodate the increased number of available acetyl CoA molecules; hence the overflow is shunted to acetoacetate formation. Actually, there is recent evidence that an increased rate of combustion of fatty acids in the liver may induce a decrease in TCA cycle activity by altering the potentials of certain oxidation-reduction systems within the liver cell.

By analogy with other biochemical processes, an accumulation of metabolic products such as ketones might be expected to inhibit the system responsible for their manufacture. In ketogenesis, such a negative feedback mechanism does not appear to operate. As Krebs and Lowenstein [*58*] have stated, "It cannot yet be explained why there is an over-production of acetyl CoA in the ketotic organism, i.e., why the formation of acetyl CoA is not controlled by the rate of its disappearance through the TCA cycle as is normally the case."

Ketosis seems to be a combined disorder in which inordinate quantities of fatty acids are delivered to the liver at a time when the routes for disposal of such acids have become constricted. For example, TCA cycle activity may be reduced [*48*], and fatty acid biosynthesis, perhaps because of a deficiency of cofactors needed in the transformation of crotonyl CoA to butyryl CoA, is greatly decreased [*59–61*].

Since the impetus for mobilization of free fatty acids is a decreasing supply of available carbohydrate, and since lipogenesis also is inhibited during carbohydrate privation (whether due to diabetes mellitus or other causes), it is difficult to design experiments to separate these two factors. However, it seems to be possible, by perfusing the liver with fatty acids, to induce increased ketogenesis even when carbohydrate deficiency is not present.

Various endocrine influences that regulate the release of free fatty acids from adipose cells have been under intensive study recently. Pituitary growth hormone (somatotropic hormone [STH]) has been found to induce mobilization of free fatty acids in normal subjects, and when this hormone is administered to animals with experimental diabetes or to patients with growth-onset diabetes, marked hyperketonemia may result [*62,63*]. Epinephrine, norepinephrine and a pituitary factor distinct from STH also promote the release of free fatty acids from fat depots, while thyroid hormone and cortisone may potentiate fat mobilization [*45*]. The mechanisms that control the rate of secretion of the various hormones concerned with the release of fatty acids are not well understood. Probably the endocrine glands concerned are responsive in some way to the carbohydrate supply, since lack of available carbohydrate in the body

always results in a shift to an economy in which fat oxidation predominates. (Fig. 5.)

Thus, in summary, the phenomena of ketogenesis and hyperketonemia have been considered as components of the adaptive response of the body to carbohydrate privation. The fact that such a response is intensified to a pathologic degree in diabetes mellitus and in certain other metabolic disorders does not invalidate the concept of ketogenesis as a normal mechanism whereby readily utilizable fuel is delivered from the liver to other tissues.

Why hepatic ketogenesis sometimes becomes uncontrolled is unknown. At least two distinct processes, both related to carbohydrate deficiency, appear to be involved in the development of hyperketonemia. One acts in the periphery to promote increased mobilization of free fatty acids, and the other acts in the liver to favor formation of an "excess" of acetyl CoA fragments, which must be disposed of by means of ketone formation.

The order in which these events occur and their interrelationships will continue to be fruitful subjects for investigation.

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Critique of the Therapeutic Usefulness of the Oral Agents in Diabetes*

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DURING the past six years oral hypoglycemic agents have been used extensively throughout the world and have been given to hundreds of thousands of persons, and many investigations have been carried out both in the laboratory and clinic in an attempt to discover the mechanism and place of action of the compounds. Consequently, a vast amount of information is now available which makes possible an evaluation of the usefulness of these agents in the management of diabetes mellitus. At the outset it must be stated that the available data, although great in bulk, are still inadequate and leave many basic questions unanswered.

DEVELOPMENT OF ORAL HYPOGLYCEMIC AGENTS

Sulfonylurea Compounds. In 1930 Ruiz, Silva and Libenson [*1*] reported that certain sulfonamide derivatives exert a hypoglycemic effect. This was again pointed out in 1942 by Janbon [*2*], Loubatières [*3*] and their co-workers in Montpellier, France, and over the years these investigators have continued to carry out studies, chiefly in animals. In their early work they used *p*-amino-benzenesulfonamido-isopropylthiodiazole (2254 RP or IPTD). Loubatières found that this compound was ineffective in completely depancreatized animals and adopted the hypothesis that the compound exerts its action by stimulating the processes involved in the secretion of insulin. In 1946 Chen, Anderson and Maze [*4*] in the United States noted that sulfanilamido-cyclopropylthiazole lowers the blood sugar of rabbits. These reports attracted relatively little attention although Houssay [*5*] described such effects in a paper published in 1950. It was not until 1954 that much in a practical way came from the studies on sulfonamide derivatives as hypoglycemic agents. At this time Franke and Fuchs [*6*] in Berlin, while testing the antibacterial activity of a new compound designated as BZ 55 (N_1-sulfanilil-N_2-n-butylcarbamid), found this substance to be active in lowering the blood sugar and began clinical trials in diabetic patients. Studies were undertaken promptly and carried on energetically by workers in Germany [*7*] and in other countries. The era of oral hypoglycemic agents began in earnest.

BZ 55 or carbutamide is a sulfonylurea compound of the structure shown in Figure 1. It was withdrawn from further use with patients in the United States late in 1956 after clinical studies had indicated an incidence of toxic side effects considered to be too high [*8*]. Its use has continued in other countries although to a less extent than formerly.

In 1955, shortly after the advent of carbutamide, a related substance, *D 860* (*tolbutamide* (1-butyl-3-p-tolylsulfonylurea); Orinase®) was introduced for clinical trial, first in Germany and then in countries throughout the world. Its structural formula differs from that of carbutamide in only one respect, the substitution of a methyl for an amino group in the para position on the benzene ring. This change results in three differences in pharmacological effect: (1) tolbutamide, weight for weight, is somewhat less hypoglycemic than carbutamide; (2) it is not antibacterial; and (3) it is less apt to cause untoward effects than carbutamide.

Somewhat later a third sulfonylurea compound was made available for use. This substance, *chlorpropamide* (N-(p-chlorophenylsulfonyl)-N′-*n*-propylurea) (Diabinese®) differs from the two sulfonylureas just mentioned in that a chlorine ion stands in the para position on the benzene ring in place of an amino or methyl group. (Fig.

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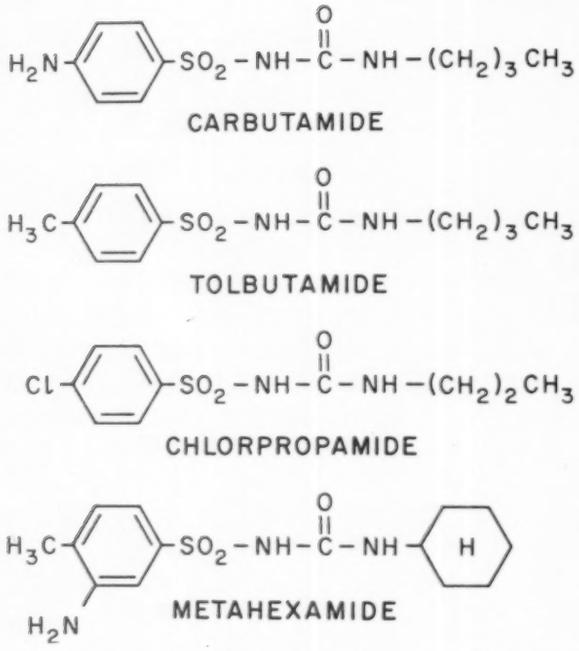


FIG. 1. Formulas of four sulfonylurea compounds.

1.) Furthermore, it is a propyl rather than a butyl compound. Milligram for milligram, chlorpropamide has a greater and more sustained hypoglycemic effect than the agents just described. This is in large part due to the fact that, whereas in the body carbutamide is acetylated and tolbutamide is carboxylated, chlorpropamide is metabolized little, if at all, and is slowly excreted in the urine unchanged. The "half-life" of tolbutamide is four to eight hours while that of chlorpropamide averages about thirty-five hours [*9*].

In the last several years attempts have been made on a large scale, particularly in the research and development laboratories of pharmaceutical firms, to discover new potent and safe chemical agents with hypoglycemic properties. To this end hundreds of compounds have been synthesized and tested in animals. A few of these have finally found their way to trial with human subjects. Among these was *metahexamide* (3-amino-4-methylbenzenesulfonyl-cyclohexylurea), the formula of which is shown in Figure 1. This compound is an effective hypoglycemic agent [*10*] but its use was attended by an unduly high incidence of toxic effects, particularly on the liver [*11,12*], so that in June 1959, after clinical trials for several months, it was withdrawn from further use in the United States.

Other sulfonylurea agents which have been or are in the stage of clinical investigation include *cyclotolhepatamide* (1-cycloheptyl-3-*p*-tolyl-sulfonylurea), a tolbutamide-related compound, and *acetohexamide* (*p*-acetyldiorthophenyl-cyclohexyl-sulfonylurea). Studies thus far have indicated that these compounds are effective hypoglycemic agents but they do not have any striking advantages over the preparations now on the market.

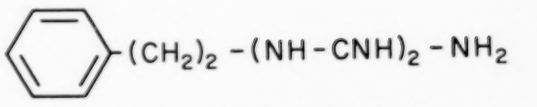


FIG. 2. Formula of phenformin (phenylethylbiguanide).

Biguanides. Entirely apart from studies on sulfonylurea compounds has been the clinical trial during the past few years of certain biguanides. They are among the guanidine derivatives but, in contrast to the substituted monoguanidines such as methylguanide and the alkylated biguanidines such as Synthalin® [*13*], they are apparently non-toxic in human subjects, at least in those amounts which can be tolerated without digestive upsets. Although it had been reported some thirty years ago that certain of the lower alkyl derivatives of the biguanides (formamidinyliminoureas) were hypoglycemic when administered orally [*14*], it was not until 1957 when Ungar, Freedman and Shapiro [*15*] published their findings that a study of these compounds was pursued vigorously. Ungar et al. found that *phenethylbiguanide* (phenformin; DBI), given parenterally or orally, lowers the blood sugar in guinea pigs, rats, rabbits, cats and rhesus monkeys. It also reduces the blood sugar of alloxan-diabetic rats, rabbits and monkeys. The structural formula of phenethylbiguanide is shown in Figure 2. Many other compounds related to phenformin have been prepared and the most promising ones distributed for clinical trial. Among these, the ones most widely used are butylbiguanide (DBB; buformin; W 37) and dimethyldiguanide (metformin; Glucophage), both employed chiefly outside the United States.

Other Hypoglycemic Agents. There are various substances which may under suitable conditions lower the blood glucose without necessarily correcting the metabolic abnormalities of diabetes. For many years various plant extracts have been used, especially in folk medicine, in the attempted treatment of diabetes [*16*]. Also of interest are the substances known as Hypoglycin A and B which have been isolated from the fruit

("ackee") of a tree, *Blighia sapida*, grown especially in Jamaica [*17*]. Although these compounds are strikingly hypoglycemic, their toxicity precludes their use with patients. Finally, mention must be made of the action of aspirin and certain other salicylates, when given in large amounts, in lowering the blood glucose of some diabetic patients [*18*]. The mechanism of action is not clear.

THE SULFONYLURIA COMPOUNDS

Mode and Site of Action. Since the present discussion is directed along clinical lines, it would be out of place to present a detailed review of the results of the extraordinarily large number of studies made to elucidate the mechanism of action of the oral hypoglycemic agents. However, since such data have a direct bearing on the rationale of trial, and particularly of long-continued use, of these substances in diabetic patients, a brief summary of current views will be given. More detailed presentations [*12,19–23*] with extensive bibliographies should be consulted for further information.

There is now fairly general agreement that the sulfonylurea compounds exert their effect chiefly by stimulating the release of insulin from the beta cells of the islands of Langerhans. The details of such action are far from clear, but that it takes place has been shown by the finding of additional insulin (or a substance with insulin-like activity) in the pancreatic vein blood following the administration of sulfonylureas [*24*]. Furthermore, there is a definite decrease in the granulation of the beta cells following the acute administration of sulfonylurea compounds [*25*].

If the sulfonylureas promote the release of insulin and exert their action through endogenous insulin, one might expect to find the known metabolic effects of insulin following their administration. However, although reported data have been somewhat discordant, most of the attempts to demonstrate increased utilization of glucose by peripheral tissues have been unsuccessful. Following administration of a sulfonylurea compound, it is difficult or impossible to demonstrate a rise in the respiratory quotient, an increase in the arterial-venous blood glucose difference, decrease in the serum phosphorus following the administration of glucose or an increase in the blood lactate or pyruvate levels following the intravenous administration of glucose [*26*]. To explain this discrepancy, the concept has arisen and gained considerable acceptance that the chief effect of the insulin released by sulfonylurea stimulation is in the liver in which it acts to reduce hepatic glucose output and thereby contribute to the decrease in the blood glucose. Insulin not bound in the liver would be free to act in peripheral tissues.

Support for the idea that the chief site of action of the sulfonylureas is in the pancreas is found in the facts that these compounds are not effective in depancreatized men or animals (except, to some extent, in the latter if maintained on a basic dose of insulin [*27*]) and that the diabetic patients who respond best are those in whom there is good evidence to suggest that a considerable capacity for insulin production persists. Conversely, in patients in whom the ability to produce insulin is limited or approaches zero, as in those with childhood diabetes a few years after onset, the drugs are not effective [*28*].

Other ways in which the sulfonylureas might exert their action have been explored with largely negative, or at least inconclusive findings. Although experiments *in vitro* have yielded equivocal results, data from studies in animals lend little or no support to the idea that, in dosage used in patients, the compounds work by a direct effect either on the liver or peripheral tissues. An early thought that the drugs act by damaging the pancreatic alpha cells and thereby inhibiting the production of glucagon has been disproved The concept that prolongation of the hypoglycemic effect seen following the administration of sulfonylureas might be related to an inhibition of insulinase [*29*] and a consequent decrease in the destruction of endogenous insulin has found no great support. Finally, the sulfonylureas appear not to oppose directly the action of the hypophysis, adrenals, thyroid or gonads and they remain active in the absence of these glands [*30*].

Selection of Patients. The sulfonylurea compounds lower the blood sugar in normal subjects and in certain middle-aged and elderly persons with "mild" diabetes, particularly in those with an insulin requirement below 40 units daily and more strictly below 20 units daily. They are not effective in children or in adults with the unstable, juvenile type of diabetes; in patients with ketoacidosis and coma or in those patients prone to such; during infections, particularly with fever; and usually during and following major surgical operations. It is the age of onset of diabetes which is important rather than the

present age of the patient. Persons with onset of diabetes above the age of forty years may be responsive whereas those with onset under that age are apt not to be. A patient aged fifty-five with mild diabetes of recent onset may well respond; another aged fifty-five with diabetes of thirty years' duration will not.

Even if these guiding principles are observed, by no means will all apparently suitable candidates exhibit a good response to the sulfonylureas. Consequently, the matter of initial selection becomes of great importance. The following considerations must be borne in mind: (1) Patients whose diabetes can be controlled easily by restriction of diet alone, particularly if they are overweight, should not be given the drugs. Obesity should not be supported with oral agents or, indeed, with insulin. (2) Patients who really need insulin should receive its benefit and not be shifted to a sulfonylurea preparation lest disaster result in the form of either acute or chronic complications. In fact, the unwise or poorly supervised use of oral agents presents a much greater potential hazard than any toxic effects which have been noted with the present market preparations. (3) Careful selection of patients will avoid to a great extent the "secondary failures" which at present are receiving considerable attention. (4) Patients given oral agents enjoy the convenience of treatment so much that, if unsatisfactory results are obtained, it is often difficult to persuade them to accept the better protection afforded by injections of insulin.

In the selection of patients for sulfonylurea treatment the usual procedure is simply to give a patient who is considered a likely candidate an adequate dose of the drug and observe in succeeding days the effects on sugar in the blood and urine. In patients who are not receiving insulin and who have no acute symptoms, this type of therapeutic trial poses no great problem. In those receiving less than 20 units of insulin daily, usually the insulin may be discontinued abruptly and an attempt made to substitute the oral agent therefor, provided close contact with the patient is maintained. In those receiving 20 to 40 units daily, the insulin dose may be reduced by 25 or 30 per cent daily or every other day while observing the effect of the sulfonylurea compound and, if a favorable response is obtained, the administration of insulin may be discontinued entirely within a few days. In those patients whose insulin requirement is more than 40 units daily, the shift to an oral agent is best attempted under hospital conditions in order to provide close observation and to detect and treat ketoacidosis at an early stage should it occur.

During the early clinical trials with tolbutamide, my colleagues and I made use of a four hour sulfonylurea response test as an aid in the selection of patients. In this test the response of the blood glucose of a fasting subject to a single large dose of a sulfonylurea compound is determined. The test, described in detail elsewhere [*20*], is considered successful and the patient a good candidate for sulfonylurea therapy if the blood glucose, regardless of the initial level, falls in four hours to 110 mg. per 100 ml. or lower. Although by no means completely accurate in its predictions, the test is satisfactory enough for clinical purposes, especially with tolbutamide. At the present time the sulfonylurea response test is used much less than formerly because clinical experience with the drugs has made it largely unnecessary. However, it remains a valuable guide in patients who seem questionable candidates for oral treatment or in those with whom a prompt decision is needed. Use of the test in these patients will help avoid most of the "primary failures" which might occur during the first month of trial of the drug and likewise many of the "secondary failures," i.e., those which occur after one month of success initially.

Criteria of Control. In the management of diabetes with oral hypoglycemic agents it is imperative that strict standards of control be established and adhered to. All too often patients are allowed to carry along with no measure of control other than urine tests. This is true even with certain clinicians who have reported their results in published articles here and abroad. In middle-aged and elderly persons who constitute likely candidates for sulfonylurea therapy, it is not enough that the urine be consistently free from sugar. In this age group the renal threshold for glucose is frequently elevated. Consequently, at the initiation of treatment and periodically thereafter, the blood glucose should be determined sufficiently often to make sure that it is usually at a satisfactory level.

A workable rule is to insist that for control to be considered "good," the majority (arbitrarily, 70 per cent) of blood glucose values be 110 mg. per 100 ml. or lower at three or more hours after the ingestion of food. "Fair" control may be said to be achieved when the majority of corresponding blood glucose values are 130 mg. per 100 ml.

or lower. In either case the twenty-four hour collection of urine will contain little or no sugar. All other results may be considered indicative of "poor" control. If, despite adherence to a properly restricted diet, patients cannot achieve good or fair control, they should be shifted to insulin. In fact, with those whose control is only "fair" it is frequently best to abandon the oral agent and shift to insulin since they represent the recruits for "secondary failures" later.

Dosage. Because of its relatively short duration of effect, *tolbutamide* (Orinase) usually must be given twice daily, before breakfast and before supper. Treatment may be started by giving one tablet (0.5 gm.) at each of these times. If this proves inadequate, as gauged by urine and blood tests, another tablet may be added to the morning dose and later, if need be, another to the evening dose, making four tablets or 2 gm. in all. Usually, if a patient is not responsive to this amount, he will not respond to larger doses. However, occasionally some additional effect is obtained by increasing the daily dosage to 2.5 or 3 gm. by giving one or two tablets before the noon meal.

In a patient who proves to be responsive to only 0.5 gm. twice daily, an attempt may be made to lower the dose to one tablet daily. If blood glucose values still continue at a normal level, trial without tolbutamide is indicated. However, the patient should be urgently advised to continue strict adherence to diet and to continue urine tests for sugar before each meal and at bedtime. If glycosuria reappears and the blood glucose level becomes abnormal then return to the oral agent should be made.

Because of its relatively long "half-life" and slow excretion from the body, chlorpropamide (Diabinese) needs to be administered only once daily. Furthermore, to avoid undue accumulation of the drug in the body with consequent prolonged hypoglycemia, the daily dosage should not exceed 500 mg. Daily amounts of 100 to 250 mg. are adequate in many patients; the smallest dose which will provide good control should be used.

Untoward or Toxic Effects. When considering a new treatment agent one must ask at least two questions: Does it work? Is it safe? It is evident that in certain persons with diabetes the sulfonylurea compounds do exert a hypoglycemic effect and so the first question can be answered with a qualified affirmative. As for toxicity, two sulfonylurea compounds which reached the stage of extensive clinical trial—carbutamide and metahexamide—were withdrawn from further use in the United States because of an incidence of toxic effects considered to be too high. In the case of carbutamide, the untoward effects, noted in 5.4 per cent of 7,193 patients for whom data were available, consisted chiefly of allergic skin responses, anorexia, nausea, vomiting, malaise, fever, leukopenia, agranulocytosis and jaundice. Eight deaths occurred under circumstances suggesting that the use of carbutamide may have contributed [*8*]. It is only fair to state that the opinion is held by many outside the United States that carbutamide is a relatively safe drug. As for metahexamide, there has been general agreement that the incidence of toxic effects particularly on the liver, is high enough to preclude its general use.

Despite the widespread use of tolbutamide during the past six years, there have been few reports of toxicity or of important side effects. Jaundice possibly related to tolbutamide has been described only rarely and then under unusual conditions with a past history of liver dysfunction. The incidence of side effects necessitating discontinuance of tolbutamide has ranged in published reports from 1.1 to 1.6 per cent [*31–33*]. Since it is metabolized quickly and does not accumulate in the body, it rarely causes symptomatic hypoglycemia and then chiefly in fasting or undernourished elderly persons with very mild diabetes.

In the early experience with chlorpropamide some few years ago, the incidence of untoward and toxic effects was considerably higher than now, apparently because of the larger doses given at a time when clinical trials were in the exploratory stage. When chlorpropamide is given in amounts of 1 gm. or more daily, some patients experience lethargy, muscular weakness, ataxia and dizziness. Other symptoms which have been described include anorexia, nausea, vomiting, abdominal discomfort, chest pains, skin rashes and leukopenia [*34,35*]. In addition, with larger doses, an occasional complication is prolonged hypoglycemia due to the slow excretion of chlorpropamide and consequent accumulation of the drug in the body [*36*]. In the last three years during which the daily dosage has been kept by most clinicians at a level no greater than 500 mg., the frequency of untoward effects has become much less. Even so, occasional cases of prolonged hypoglycemia have been

TABLE 1
PROBABLE CAUSES OF SECONDARY FAILURE TO TOLBUTAMIDE IN 1,965 PATIENTS

Probable Cause of Failure	Cases of Failure	
	No.	Per cent
Inadequate dosage of drug	163	8.3
Poor initial selection	110	5.6
Poor adherence to diet	68	3.5
Temporary failures	18	0.9
True drug failures	73	3.7
	432	22.0

reported in sensitive persons [*37*]. Even with the smaller doses now used, there are occasional reports of allergic response to the drug manifested by a skin rash, fever, eosinophilia and a rise in the serum alkaline phosphatase level. Overt jaundice may appear. The jaundice is usually of a benign, reversible type; liver biopsy usually reveals intracanalicular bile stasis. Most patients who will exhibit drug idiosyncrasy of chlorpropamide will do so within five or six weeks after beginning treatment.

Bloodworth and Hamwi [*38*] made a histologic study of tissues obtained at autopsy in twenty diabetic patients who had been treated with sulfonylurea compounds for varying lengths of time. They reported the finding of widespread microgranulomas, to a degree greater than in non-treated subjects, most often in the liver and kidneys but also frequently in the heart, adrenals and gastrointestinal tract. There were, in addition, diffuse infiltrates in the heart (myocarditis) and kidneys (interstitial nephritis). They regarded the changes as probable evidence of a hypersensitivity reaction. In no case could the abnormalities be related either to symptoms during life or to the principal cause of death.

Results of Treatment. There is general agreement that among patients with mild diabetes of maturity-onset type, the diabetic condition can be satisfactorily controlled in 50 to 70 per cent by means of appropriate doses of a sulfonylurea compound. Mehnert, Camerini-Dávalos and Marble [*31*] reported that in a selected group of 772 patients at the Joslin Clinic, good control of hyperglycemia and glycosuria was obtained in 52.6 per cent and fair control in 18.5 per cent. Lennon, Engbring and Engstrom [*39*] noted good control in 76 per cent of 104 selected patients treated with tolbutamide. The reported data of other clinicians agree in general with these findings and it would not be profitable to present here even a summary of reports in the literature. In fact, it is most difficult to compare the findings and impressions of one clinician of those with another because of variation in the many factors which influence the outcome. Among such factors are the selection of patients, place of the clinical trial (i.e., whether in an outpatient department or under more carefully controlled conditions in a hospital), type of diet, frequency and interpretation of results of determinations of urine and blood glucose, adequacy of data and standards of control. Unfortunately, as mentioned earlier, in many reports no mention is made of blood glucose standards or values obtained.

At the present time the matter of the so-called "secondary failure" to sulfonylurea compounds is provoking much discussion. DeLawter et al. [*40*] have reported their results with 200 patients who received tolbutamide for periods up to three years. Following the first year of use, the secondary failure rate was 29.5 per cent and the monthly failure rate averaged about 3 per cent of the patients treated each month. The authors speculate that with continued use of tolbutamide, practically all persons will become unresponsive to the drug after the passage of several years. However, the experience of Marble and Camerini-Dávalos [*41*] suggests that this conclusion may not be warranted. They found that of 1,965 patients treated with tolbutamide at the Joslin Clinic for periods up to five years, 432 or 22 per cent experienced secondary failures. Analysis of the probable causes for the secondary failures resulted in the breakdown shown in Table 1. This represents a preliminary analysis, and further study during the coming months may reveal that among those listed as given inadequate dosage (142 of the 163 had received only 1 gram or less daily), there may be some with "true" drug failure. The factor of poor initial selection is an important one and emphasizes the need for care in choosing patients for oral therapy if one hopes to achieve success over years of time. As for adherence to diet, any clinician with experience in the use of oral hypoglycemic agents knows that care in following a restricted diet is imperative if satisfactory control is to be achieved and maintained.

In general, those patients who are responsive

to tolbutamide are also responsive to chlorpropamide and *vice versa*. It has been stated that some patients in whom only a partly successful result is obtained with tolbutamide, or in whom a secondary failure to this agent has taken place, may respond more satisfactorily to chlorpropamide.

THE BIGUANIDES

Mode and Site of Action. In the five years since the first systematic clinical trials of phenformin (phenethylbiguanide; DBI) there have been many studies not only to evaluate the effect of this compound and its analogues in the treatment of diabetic patients but also to discover the mechanism and place of action of these agents. The data at times have been contradictory and more often the interpretation of results by different workers has been divergent. A great deal is known about what the biguanides will do in *in vitro* experiments but as to the exact means by which they bring about a lowering of the blood glucose in certain diabetic patients is still a matter of controversy. Strange and unexplained is the fact that whereas the biguanides lower the blood glucose in normal animals, they do not do so in the non-diabetic human subject [*42*].

Studies *in vitro* by Williams and his associates [*43*] showed that phenformin causes an increase in uptake of glucose by the rat hemidiaphragm but instead of an increase there is a decrease in glycogen and an increased lactic acid production. They interpreted their findings as suggesting that the increased glucose uptake might result from inhibition of oxidative activity rather than from a direct stimulation of anaerobic glycolysis. From these and other data they concluded that the biguanides may produce a lowering of blood sugar by means of a inhibitory effect on certain respiratory enzymes. They suggested that the primary site of interference was at the cytochrome oxidase level whereas Wick, Larson and Serif [*44*] concluded that it was at some point prior to cytochrome C in the succinic oxidase system. When phenformin is added to liver preparations *in vitro*, a reduction in glycogen content and increased production of lactic acid are observed [*45*].

Despite the findings in *in vitro* studies which appear to indicate that phenformin may inhibit electron transport and enzymatic oxidation in muscle, adipose tissue and liver, Ungar, Psychoyos and Hall [*46*] have expressed great doubt as to the validity of the idea that the hypoglycemic action of phenformin arises from inhibition of tissue oxidation. They state that certain biguanides which have a hypoglycemic effect *in vivo* have little or no inhibitory effect on tissue oxidation *in vitro*, and vice versa. Furthermore, it must be pointed out that the amounts of biguanide used in the *in vitro* studies have been much greater than those which lower the blood glucose in certain diabetic subjects. It would appear that in some way the biguanides increase glycolysis along the anaerobic pathway, but the details of this and subsequent metabolic events remain unclear.

Selection of Patients. It is evident from the foregoing discussion that the biguanides act in a manner unrelated to that of insulin or of the sulfonylureas. As mentioned at the beginning of this article, Ungar, Freedman and Shapiro [*15*] early showed that phenformin may lower the blood glucose in a wide variety of animals including those with alloxan diabetes. In human diabetic patients, phenformin has a hypoglycemic effect in certain middle-aged and elderly persons to an extent roughly similar to that of the sulfonylurea compounds. Furthermore, it is capable of some blood glucose-lowering effect in certain young patients with the unstable, growth-onset type of diabetes.

Despite this wide range of effectiveness, great care must be taken in the selection of patients for trial of phenformin. Although it may yield satisfactory results when used alone in older patients with mild diabetes and a low insulin requirement (under 20 units daily), provided a diet restricted in carbohydrate is followed, it must be employed with great care in those with higher insulin requirements, and particularly in children and others who are prone to ketosis. In such patients, if it is the decision of the physician to try phenformin, the drug should be used as a supplement to insulin and not as the main feature of treatment. If the reverse is allowed to take place and insulin used merely to supplement phenformin, disaster may result in the form of ketoacidosis [*47*].

Criteria of Control. In those patients in whom phenformin is used as the sole hypoglycemic agent along with a restricted diet, the standards of control should be as high and as rigorously maintained as outlined in the earlier discussion on the sulfonylureas. In those patients in whom phenformin is used as a supplement to insulin in an attempt to lower the amount of insulin

necessary and thereby make the course of unstable diabetes somewhat smoother, it is much more difficult to set up arbitrary standards and interpret results. In fact, it is difficult to document the considered opinion of various clinicians that the use of a phenformin supplement improves the course of unstable diabetes [*48*]. Certainly the minimum requirement should be that with the addition of phenformin the amount of sugar in the twenty-four hour collection of urine and the general trend of the blood glucose should be lower and less fluctuating than under comparable conditions without phenformin.

Dosage. Using 25 mg. tablets, treatment may be initiated by giving one tablet before breakfast and before supper. If well tolerated and if a larger amount seems necessary, a third tablet may be given before the noon meal and then one added at bedtime. In this way the dose may be increased gradually until it consists of two tablets (50 mg.) before each meal and one or two tablets at bedtime (175 to 200 mg. daily in all). Usually, in responsive patients, this amount will suffice and will be about as much as can be given without digestive disturbances.

For some time phenformin time-disintegration (DBI-TD) capsules, 50 mg. and 100 mg., have been available for clinical trial. These lengthen the duration of effect so that the drug usually needs to be given only twice daily.

Untoward or Toxic Effects. Although results of *in vitro* studies suggest, as has been discussed earlier, that the biguanides inhibit oxidative processes and favor anaerobic glycolysis, human diabetic patients appear to tolerate these compounds in therapeutic doses for years without apparent harmful effects. Since 1956 thousands have been given these drugs without any report of lasting tissue or organ toxicity so far.

The occurrence of ketonuria in certain subjects receiving biguanides has long been recognized, but in most articles reporting the use of phenformin in large groups of patients it is given only scant mention. There has been general agreement that acetonuria is related to inadequate carbohydrate utilization and will disappear with an increase of carbohydrate in the diet and perhaps increase in insulin dosage along with decrease in the dose of phenformin. Apparently symptomatic ketoacidosis has not been a significant problem in the experience of most clinicians. However, Walker and Hannah [*47*] noted ketonuria in thirty-four (29.1 per cent) of 117 patients receiving phenformin; an alkali reserve of less than 20 mEq. per L. was found in thirteen. Their experience emphasizes the fact that, particularly in patients with growth-onset and/or unstable diabetes, insulin must form the basis and mainstay of treatment [*49*], and phenformin, if given, must be used as a supplement.

A common finding has been that when the daily dose of phenformin is in excess of about 200 mg., unpleasant side effects occur. These consist of anorexia, nausea, abdominal distress, vomiting and diarrhea. The upper limit of tolerance varies from person to person: in some, it may be 150 mg. daily or even less; in others it may extend to 300 mg. or even higher. Such untoward effects promptly disappear with lowering of the dose or discontinuance of the drug.

Results of Treatment. Beginning in 1957 Krall and colleagues [*23*] have reported at intervals their results with biguanide compounds. In one series of 244 patients, which included many in whom the diabetic condition was unstable and difficult to control, a significant blood sugar-lowering effect was observed in 88 per cent [*50*]. Krall regards phenformin as particularly useful in smoothing the course of patients with unstable diabetes. In certain patients of this type phenformin may decrease the insulin requirement by as much as 25 to 50 per cent.

In 1959 Pomeranze et al. [*51*], reporting their results in 206 patients who had received phenformin for periods up to two years, expressed their opinion that phenformin is an effective hypoglycemic agent in all types of diabetic patients. Among the 206 patients, 128 (62 per cent) were under treatment which the authors considered successful at the time of their report. Barclay [*52*] stated that fair to excellent control was achieved in 88 per cent of 104 patients aged four to eighty-three years who had been treated with phenformin with or without the concomitant use of insulin.

PLACE OF ORAL AGENTS IN THE MANAGEMENT OF DIABETES

How useful are the oral hypoglycemic agents in the treatment of diabetes? Are they truly antidiabetic or only hypoglycemic agents? Has their introduction resulted in better or worse control of diabetes in the diabetic population at large? Do they deserve a lasting place in the management of diabetes? With long-continued use may harmful effects appear which have

not been evident to date? Over the years will their use prevent to a greater or lesser degree the late complications of diabetes in the vascular and nervous systems? These pertinent questions are difficult to answer and some must obviously await the passage of time.

It is a matter of every day experience that in a high percentage of selected patients with mild diabetes of maturity-onset type, the urine can be kept free from sugar and the blood glucose at normal or near normal levels (110 mg. per 100 ml. or lower at three or more hours after the ingestion of food) by the giving of sulfonylurea compounds, provided that a diet restricted in carbohydrate is consistently followed. Success in use of these compounds appears to be in direct relation to the ability of the beta cells of the pancreas of the subject to respond to stimulation. Many patients, even among those who would appear to be excellent candidates, do not respond well to the sulfonylureas. One constantly sees examples of the misuse of sulfonylurea compounds. Patients are started on the oral agents when they really need insulin for good control of diabetes and are then maintained, often for long periods, on the drugs despite hyperglycemia and even glycosuria. In some instances few or no determinations of the blood glucose are made and urine tests are performed infrequently and then only after an overnight fast. The most flagrant examples of the unwise use of the sulfonylurea compounds are seen in the attempted treatment of persons with onset of symptomatic diabetes under the age of thirty years. Such patients may, indeed, at times appear responsive to oral therapy during a period of remission in the first weeks or months after onset of diabetes and initiation of treatment. However, they almost invariably require insulin and unless they are seen frequently by the physician and their treatment carefully supervised, ketoacidosis eventually supervenes.

These comments do not apply to carefully conducted programs of clinical investigation such as that of Fajans and Conn [*53*] who have shown that the carbohydrate tolerance of young persons with mild asymptomatic diabetes may be greatly improved by the administration of tolbutamide. Somewhat similar findings were noted in alloxan-subdiabetic rats by Weber et al. [*54*]. After twelve to fifteen months of tolbutamide feeding the glucose tolerance was significantly better than that of control animals. These results lend hope to those working on the problem of the prevention of diabetes.

Some have criticized the use of the sulfonylurea compounds with the conviction that, although they may lower the blood glucose, they do not correct the metabolic error of diabetes. However, there is good reason to believe that if a diabetic person is maintained on a diet which is truly adequate in protein, vitamins, minerals and calories, the level of the blood glucose is a reliable and easily measured index of the status of underlying metabolic processes.

As for toxicity, the use of tolbutamide is attended by remarkably few untowards effects of any significance. Chlorpropamide in large dosage may lead to difficulties, but if the amount given daily does not exceed 500 mg. the incidence of important side effects is quite low. It is true that drug hypersensitivity appears to be more common to chlorpropamide than to tolbutamide and that, even with doses in the approved range, an occasional instance with dermatitis, fever, evidences of hepatic dysfunction and even jaundice may appear within five or six weeks of starting therapy with the drug. The jaundice has been almost always of the "benign," cholestatic type. However, although this type of side effect is a possibility, the risk is not great since it is stated that of over 100,000 persons who have received chlorpropamide, only thirty-three cases of jaundice, eight or nine of which are not well documented, have come to the attention of the manufacturer and most of these occurred two or more years ago on doses larger than now recommended [*55*]. There are as yet no data available regarding the incidence of chlorpropamide-induced jaundice in patients receiving present day dosages.

There is at the present time a considerable difference of opinion among clinicians as to the place and value of the biguanides in the treatment of diabetes. There are those who look with disfavor on any use of these compounds and there are others who regard them as "the oral therapy of choice in the management of diabetes" [*52*]. In the majority of selected middle-aged and elderly persons with mild diabetes the level of the blood glucose may be kept at a satisfactory level by the use of phenformin. The physician must choose between phenformin and one of the sulfonylurea agents. There are many young patients in whom the use of phenformin as a supplement to insulin causes the insulin requirement to fall significantly and apparently make

the course of the unstable diabetes less fluctuating. In both old and young patients, the administration of phenformin should be continued only if truly good results are obtained as shown by periodic tests of the urine and blood.

Phenformin has occasionally proved useful in older patients in whom a sulfonylurea compound alone has produced "fair" but not "good" results. The addition of phenformin in dosage of 50 to 150 mg. daily to the treatment schedule may result in much better control. Combined treatment has been commented upon particularly by Beaser [*56*] and Mehnert [*57*].

SUMMARY

When used in connection with a carefully restricted diet and in the absence of ketoacidosis, the sulfonylurea compounds provide "good" or "fair" control of hyperglycemia and glycosuria in 50 to 70 per cent of patients with onset of stable diabetes above the age of forty years and with a daily insulin requirement of 20 units or less. The mechanism of action appears to be that of stimulation of release of insulin from the pancreatic beta cells and the effect of such insulin first in the liver with decrease in the hepatic glucose output. Action in the periphery of insulin not bound in the liver is not precluded.

The frequency of significant untoward effects from tolbutamide is small, and the drug is well tolerated by almost all persons. Chlorpropamide is likewise relatively free from side effects if used in dosage no larger than 500 mg. daily. Doses of 1 gm. per day or greater may lead to prolonged hypoglycemia due to the slow excretion of the drug and excessive accumulation in the body. Jaundice of reversible, cholestatic type may occur, apparently on the basis of hypersensitivity, but very few cases have been reported during the past three years when smaller doses have been the rule.

Secondary failures (failure despite success initially for at least one month) in the Joslin Clinic series of 1,965 patients treated with tolbutamide for periods up to five years amounted to 22 per cent. However, poor adherence to diet, poor initial selection of patients and inadequate dosage of the drug seemed responsible for the bulk of the failures.

The biguanides such as phenformin seem to be effective in the same group of middle-aged and elderly patients who are responsive to the sulfonylureas. As a supplement to insulin, they may reduce the insulin requirement and assist in the management of young patients and others with unstable diabetes. The modes and site of action are not clear although results of *in vitro* studies suggest inhibition of oxidative metabolism and increase in anaerobic glycolysis.

Amounts of phenformin greater than 200 mg. daily often cause anorexia, nausea, vomiting and diarrheas. However, no instance of true tissue or organ toxicity has been reported.

Great care should be taken in the selection of patients for treatment with oral hypoglycemic agents. Furthermore, patients must be kept under close supervision and the degree of control evaluated by frequent tests of the urine and blood for sugar. It is imperative that there be strict adherence to a diet which is nutritionally adequate but restricted in carbohydrate. The aim must be to maintain "good" control defined arbitrarily as that state in which, with the patient on an adequate diet, the majority of blood glucose values, both fasting and non-fasting, at three or more hours after the ingestion of food are 110 mg. per 100 ml. or lower.

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End of Symposium

Clinical Studies

The Role of the Kidney in Human Hypertension*

I. Failure of Hypertension to Develop in the Renoprival Subject

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THE participation of the kidney in the maintenance of increased systemic arterial tension has been well established since the observations of Tigerstedt and Bergman [*1*] in 1898 and the classic experiments of Goldblatt [*2*] in 1934. A vast amount of experimental and clinical data attest to the fact that the kidney in some way is responsible for arterial hypertension, but precisely in what way or which several ways is a moot question. The various roles assigned to the kidney in the production and maintenance of hypertension may be roughly classified as follows (Fig. 1): (1) The affected kidney releases into the systemic circulation a pressor substance or the precursor thereof which acts directly upon the small arterioles to produce hypertension [*3*]. (2) The affected kidney fails to metabolize a pressor substance formed in the kidney or elsewhere which is then released to produce hypertension [*4*]. (3) The affected kidney fails to excrete a pressor substance of renal or other origin [*5*]; in each case the substance remaining unexcreted or unmetabolized may be a compound which is not pressor in itself but which sensitizes the vasculature to other normally occurring pressor stimuli [*3–5*].

The role of excess sodium ingestion in aggravating or even producing hypertension through any of these mechanisms has been documented in many experimental and clinical conditions [*6*]. It seems probable that the role of sodium in this regard is closely linked with that of water and potassium, and that the balance and distribution of electrolytes between cells and extracellular fluid may be as important as net balance for any one electrolyte [*7*]. The adrenocortical steroids, which are closely linked with mineral and water balance, are thought to participate similarly [*8,9*]. It has been suggested also that in the dog the affected kidney in some way modifies the baroreceptors of the carotid sinus so that they are "reset" at a higher level, thus actually maintaining the hypertension rather than buffering it [*10*]. Braun-Menendez [*11*] believed that a growth-stimulating substance arising in or mediated by the anterior pituitary promotes increase in renal mass. When, because of interference with the renal

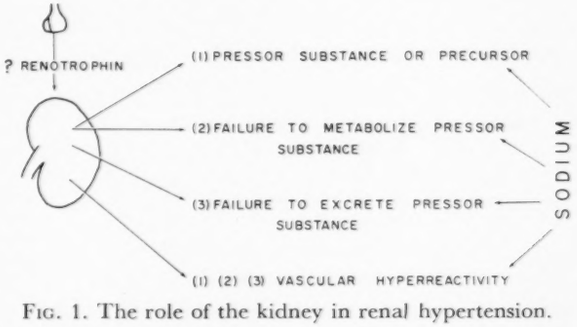


FIG. 1. The role of the kidney in renal hypertension.

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blood supply or because of renal disease, the kidney is unable to respond to this trophic factor, the consequent disequilibrium results in the development of hypertension.

Evidence from human studies bears out the importance of the role of the kidneys in hypertension but less precise experimental data are available for any of the several theories previously mentioned. Except in acute hypertension, neither renin nor any of the other renal pressor substances has been found in significant amounts in the human subject. It is possible, however, that they may be present in small amounts which may be sufficient to produce the hypertensive pattern because of hypersensitivity of the vasculature. Certainly hypertension is common in many forms of bilateral chronic renal disease, and the cure of hypertension in man following the removal of one diseased kidney or re-establishment of its normal blood supply is well documented.

Because of some notable species differences, interpretation of experimental data obtained from animals is difficult. In man the lack of experimental evidence, as well as possible species differences, further confuse analysis of the role of the kidney in hypertension. It would seem important, therefore, to study the response of the blood pressure in man totally deprived of renal tissue, and equally important to have evidence on the effect of transplanting a single kidney into a patient suffering from chronic renal hypertension and to make observations both before and after removal of the diseased kidney. The present paper will deal with observations on renoprival hypertension in human subjects. A subsequent paper [*12*] will consider the effect on hypertensive patients with chronic renal disease of successfully transplanting a normal kidney. A preliminary report of these studies appeared in 1959 [*13*].

The presence of hypertension in animals totally deprived of renal tissue (renoprival hypertension) was first described by Harrison [*14*] who found that hypertension occasionally develops after bilateral nephrectomy. In his experiments two of twelve dogs became hypertensive on the second and third day, while nine were hypotensive within the first forty-eight hours after nephrectomy. Renoprival hypertension was subsequently described in parabiotic rats by Braun-Menendez and von Euler [*15*]. In 1949 Grollman and his colleagues [*16*] recorded the presence of hypertension in bilaterally nephrectomized dogs maintained with the artificial kidney, and further observations were made on such animals maintained by intermittent peritoneal irrigation [*17*].

The difficulty in keeping such animals in fluid balance and the effects of excess sodium and water administration upon blood pressure response have been stressed. The failure of others to find hypertension in the nephrectomized dog [*18*] has been attributed [*23*] to lack of careful attention to hydration and sodium balance by previous investigators. This has been denied by the original investigators who found hypertension in nephrectomized dogs in the absence of excess sodium administration [*17*], and who believed failure to observe renoprival hypertension was due to "under hydration" [*19*]. The effects of total absence of renal tissue upon the blood pressure of man have not yet been reported. Such observations might be feasible since it is now possible to sustain the human subject for long periods in the total absence of functioning renal tissue [*13*], and to evaluate more carefully in man the effects of the variables which have led to disagreement in the interpretation of such studies in the dog. It is the purpose of this paper to report observations on the blood pressure of three human patients accidentally deprived of a single functioning kidney and kept alive for a period of twenty-four to sixty-two days thereafter. One additional patient was subjected to elective bilateral nephrectomy prior to the successful transplantation of a normal kidney from an identical twin and was studied in the renoprival state for ten days prior to the placing of the kidney isograft.

CASE REPORTS

CASE 1. A twenty-six year old white salesman (J. K., PBBH, 5H843) had been in good health until he had a dull pain in the lower right abdominal quadrant for which he was admitted to another hospital. Because of a palpable fullness in the lower right portion of the abdomen exploratory laparotomy was carried out and a large pelvic kidney was removed. Later examination revealed absence of renal tissue on the contralateral side. He was transferred to the Peter Bent Brigham Hospital on May 11, 1955, four days after nephrectomy. On admission to the hospital he was acutely ill. His blood pressure was 164/90 mm. Hg, weight 67.3 kg., serum sodium 129 mEq./L. For the first week he was maintained on a conservative regimen of intravenous fluids containing hypertonic glucose solution. The intake was sodium- and protein-free. On this regimen he lost

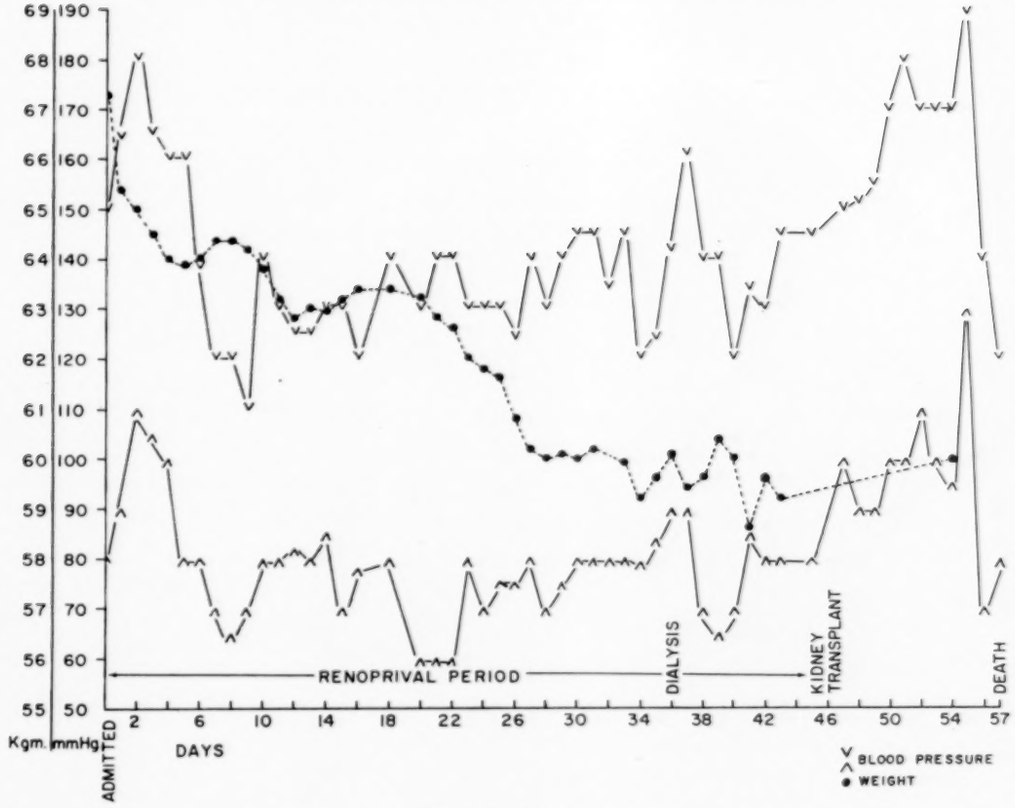


FIG. 2. Case 1.

3.5 kg. The serum sodium level rose to 135 mEq./L. The blood pressure for the first week ranged between 164/90 and 180/118 mm. Hg.* These values for his hospital course are plotted in Figure 2. On May 12, 1955, the blood norepinephrine concentration was 4.9 μg./L. of plasma. (Normal subjects studied by the same technic [*20*] were found to have 2 plus or minus 1.2 mg./L. and a normotensive patient with the same degree of azotemia 2.8 mg./L.) He was treated with hemodialysis (artificial kidney) on May 19 and June 14. On June 22 a renal homograft was performed with a kidney taken from a cadaver. This transplant never attained measurable function, but following transplantation there was a rise in blood pressure to a peak of 190/130 mm. Hg. Death occurred on the fifty-seventh hospital day; eleven days after transplantation.

Comment: The renoprival period in this person lasted for forty-six days during which time he was treated three times with the artificial kidney. The weight curve and the clinical findings suggest overhydration on admission and this is corroborated by the serum sodium of 129 mEq./L. which, in our experience with patients in acute renal failure, has most frequently been due to excessive water administration [*21*]. On May 17, the tenth hospital day, at a time when the blood pressure was 180/100 mm. Hg, plasma volume measured by the dilution of Evans blue dye was 45 ml./kg. of body weight; the upper limit of normal for this measurement is 46 ml./kg. With fluid restriction a rise in serum sodium levels occurred and the body weight dropped. Concomitant with this the blood pressure fell, reaching 140/80 mm. Hg on the tenth day. For the subsequent twenty-five days the maximum blood pressure was 145/80 mm. Hg and only three values above 140/80 mm. Hg were recorded. During this time the body weight showed a steady fall which is consistent with adequate hydration in the face of inadequate caloric intake [*21*].

During his hospital course the patient was maintained on a diet which was essentially sodium- and protein-free, consisting largely of intravenously administered dextrose and water. During certain periods he was allowed small

* The blood pressure readings listed in this study and the subsequent studies are the highest blood pressures recorded on the nurses' hourly charts. These were checked at frequent intervals by the attending physicians and by us.

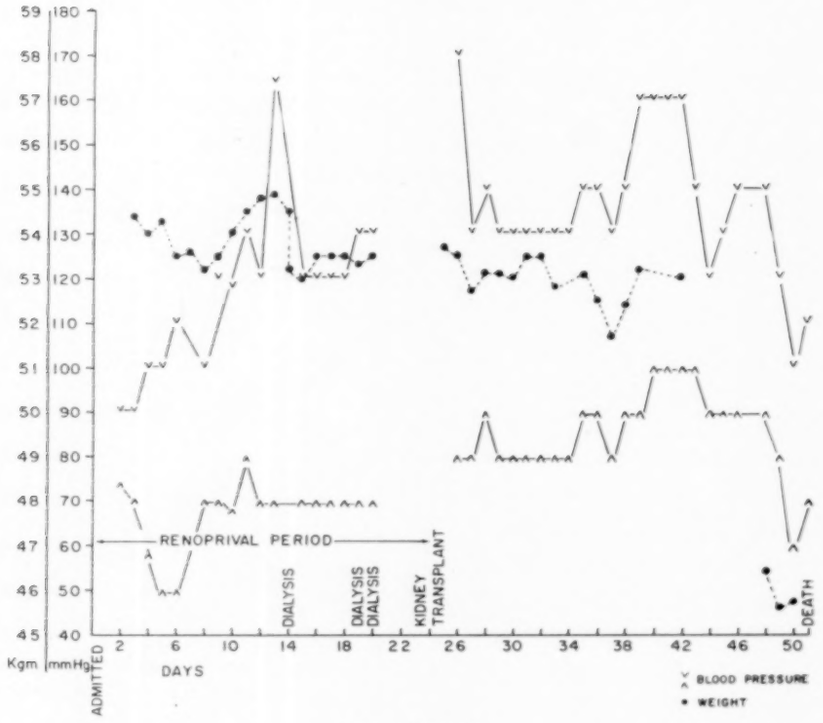


FIG. 3. Case II.

amounts of toast, salt-free butter, tea with sugar, honey, lettuce and mayonnaise, and 30 cc. of whiskey per day in ginger ale. Changes in sodium and protein intake therefore could not have been a factor in his clinical course. He was treated also with testosterone propionate, 50 mg. daily, and from the first to the tenth day with chlorpromazine. The role of the latter in depressing blood pressure is to be considered. However, its administration and dosage did not in any way relate to the changes in blood pressure which occurred. The plasma norepinephrine values seemed to be high, but the results of chromatography suggest that substances other than norepinephrine may have contributed to these values.

Our conclusion is that during the renoprival period, hypertension did not occur except in the presence of excess hydration. The transplantation of a vascularized but non-functioning kidney resulted in an increase in blood pressure. This is probably significant despite the fact that during the period after the transplant the body weight did not decrease as previously, again suggesting some excess hydration. Autopsy showed a heart which was of essentially normal size, although there was minimal dilatation of the left ventricle. There was no evidence of myocardial hypertrophy. The adrenal glands were normal and no changes were noted microscopically in the small arteries or arterioles.

CASE II. A thirty-one year old white woman (G. L., PBBH, 3M330) was admitted to the Peter Bent Brigham Hospital on March 16, 1958, one day following the removal in another hospital of what proved to be a solitary cystic pelvic kidney. On admission she appeared well oriented and nourished, not acutely ill. Her pulse was 120, blood pressure 104/70 mm. Hg, weight 54.4 kg. The pertinent points of the clinical course are shown in Figure 3. The patient was treated with a conservative regimen of protein, sodium and water restriction, and with hemodialysis with the artificial kidney. Her blood pressure remained normal throughout the first thirty-five days of her hospital stay, fluctuating around 120/80 mm. Hg. (Fig. 3.) On the twelfth hospital day the blood pressure was 120/70 mm. Hg. The patient's response to the infusion of norepinephrine, to cold pressor stimuli and to the Valsalva maneuver was within normal limits. The serum sodium concentration ranged between 135 to 138 mEq./L. for the first twelve hospital days and fell to 128 mEq./L. on the thirteenth day, at which time it was returned to 138 mEq./L. by hemodialysis. On the twenty-first hospital day she was given 600 r of whole body radiation and the following day bone marrow was infused from multiple donors. On the twenty-third hospital day a kidney homograft was

performed. No urine was elaborated until the thirty-fifth hospital day. On the thirty-fourth hospital day the blood pressure was 140/90 mm. Hg, on the thirty-eighth hospital day it rose to 160/100 mm. Hg. On the thirty-ninth hospital day the urine volume was 515 ml./twenty-four hours, on the forty-third day 1,750 ml. On this day, however, she suffered a generalized convulsion with a temporary rise in blood pressure to 200/100 mm. Hg, which was not maintained. At this time a rigorous program of dehydration was instituted, with a drop in weight and a drop in blood pressure to 120/90 mm. Hg. No further convulsions occurred. The urine volume, however, decreased progressively. The subsequent course was one of increasing uremia complicated by agranulocytosis, thrombocytopenia and hemorrhage, and diffuse infection with Escherichia coli. Death occurred on the fifty-first hospital day. Details of this case have been reported elsewhere [*22*].

Comment: The total renoprival period in this patient was twenty-four days prior to the performance of a renal homograft. During this period only one blood pressure reading of more than 140 mm. Hg systolic was obtained. This was coincident with a weight gain of 2 kg. over the preceding six days, which for this anuric patient represented definite overhydration. Dehydration with the artificial kidney on the fourteenth day resulted in a decrease in weight and a concomitant decrease in blood pressure. Her response to the infusion of a standard dose of norepinephrine and to the cold pressor test was quite normal. There was no overshoot following the Valsalva maneuver. These observations suggest a lack of vascular "hyperactivity" during this phase of the renoprival period. It is of interest that there was some increase in blood pressure ten days following the transplantation of a kidney and that this increase in blood pressure reached its peak at the point of maximum function of the homograft. This peak in blood pressure rise was accompanied by generalized convulsions. Two days prior to this convulsion the patient had had frank signs of tetany with a positive Chvostek sign and carpopedal spasm. This had been treated vigorously with an intravenous injection of calcium, with little effect. Even more important, perhaps, is the fact that two days prior to the convulsive episode the weight was 51.7 kg. and on the day of the convulsion it was 53 kg., suggesting that excess hydration had contributed both to the rise in blood pressure and to the convulsions. A dehydrating regimen subsequently resulted in both decrease in weight and blood pressure. It is difficult to evalute the effects of the severe infection, of the thrombocytopenia and of radiation upon blood pressure changes subsequent to the renal homograft. However, prior to these complicating features this patient's blood pressure remained normal in the total absence of functioning renal tissue with the exception of one rise which appeared to be correlated with overhydration.

Case III. An eleven year old boy (N. W., PBBH, 3M994) fell from a merry-go-round, injuring his right side. Because of hematuria and the findings of a mass in the right flank, a large right kidney was removed. He became anuric following this and was transferred to another hospital in Sweden* where diligent search, including exploration, failed to reveal other renal tissue. During a thirty-one day period he was treated with conservative management including fluid restriction and a high carbohydrate intake low in protein and potassium. During the thirty-one day period three dialyses with the artificial kidney were performed. After thirty-one days he was transferred to the Peter Bent Brigham Hospital. The blood pressure on admission to the second Swedish hospital was 120/80 mm. Hg. Thirty-one days later, on transfer to the Peter Bent Brigham Hospital the blood pressure was 112/58 mm. Hg, serum sodium 138 mEq./L., nonprotein nitrogen 155 mg./100 ml., potassium 5.2 mEq./L., hematocrit 21 per cent. On admission to the second Swedish hospital the weight was 38.3 kg., and on admission to the Peter Bent Brigham Hospital 37 kg. The patient was somewhat drowsy but well oriented and able to take nourishment orally. He remained in the Peter Bent Brigham Hospital for another thirty-one days before he died from infection. On the sixth Peter Bent Brigham hospital day he was treated with 700 r of whole body radiation in preparation for marrow grafting and a possible kidney transplant. During the thirty-one day period five dialyses with the artificial kidney were performed. The highest recorded blood pressure during the first eleven days at the Peter Bent Brigham Hospital was 135/100 mm. Hg with an average of 120/70 mm. Hg. (Fig. 4.) On the nineteenth day in Peter Bent Brigham Hospital he had a grand mal seizure at which time the blood pressure rose to 140/110 mm. Hg. At this time the body weight was 34.4 kg., whereas six days previously it had been 34.6 kg. On the following day he was dialysed with the artificial kidney in an attempt to diminish excess hydration. A 1.6 kg. weight loss was accomplished by this maneuver. The blood pressure fell to 124/88 mm. Hg, thereafter the highest recorded blood pressure until death was 134/74 mm. Hg. The body weight the day before death was 36 kg.

* This patient was studied through the courtesy of Dr. Nils Alwall, Lund, Sweden and his medical care in Sweden was accomplished by Dr. Alwall.

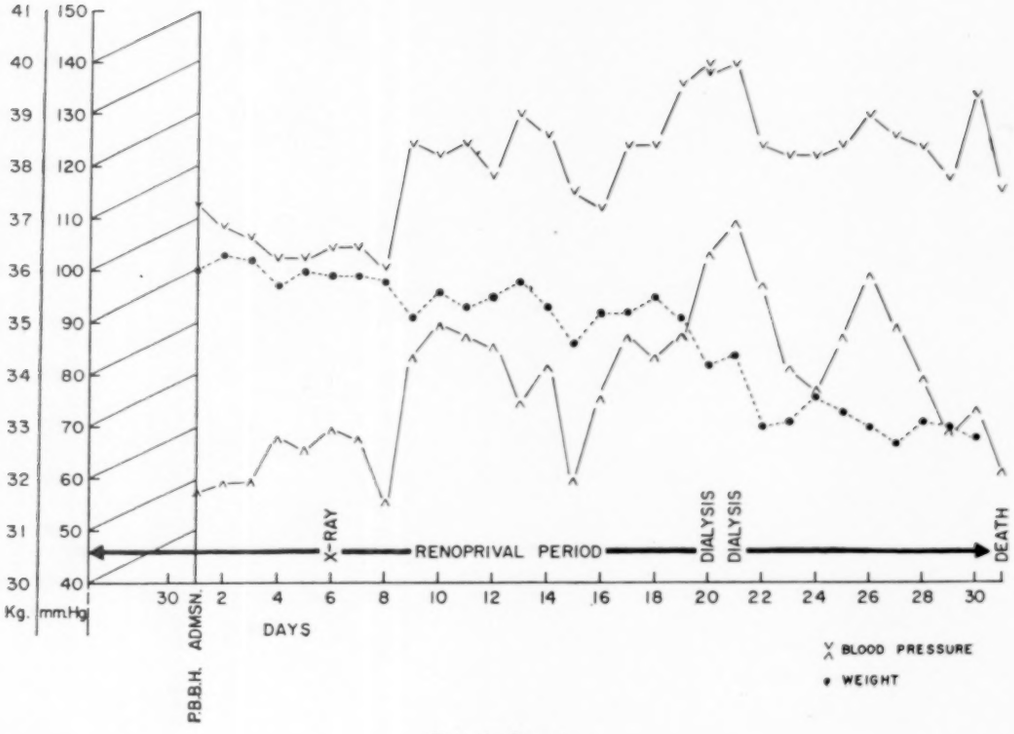


FIG. 4. Case III.

Comment: This patient was essentially normotensive during a two-month period without any renal tissue. Occasional transitory rises in blood pressure seemed to be attributable to overhydration, as evidenced by the drop in blood pressure and cessation of convulsions following a 1.6 kg. weight loss effected by ultrafiltration with the artificial kidney. Sodium intake was minimal and serum sodium within normal limits (range between 138 to 146 mEq./L.) between the thirty-first and forty-ninth renoprival day, during which the blood pressure was within normal limits. Protein intake was less than 20 gm. per day. The effects of administered antibiotics, sorbitol, gamma globulin, Dilantin® and sodium amytal could not be correlated with any change in blood pressure. Hydrocortisone, 150 mg. per day, administered during the last six days of life had no effect upon the blood pressure. In this patient a prolonged period without renal tissue was not associated with a rise in blood pressure except transitorily when it was correlated with apparent excess hydration.

During the last half of his hospital stay the effects of radiation, thrombocytopenia, leukopenia and anemia may perhaps have played a role in preventing renoprival hypertension. However, these were not factors during the first thirty-one days without renal tissue and without hypertension.

CASE IV. This twenty-seven year old white man (H. R., PBBH, 3N783) had been well until the middle of February 1959, at which time acute glomerulonephritis developed. His course thereafter was steadily downhill, with progressive uremia and oliguria. Because of this he was treated with the artificial kidney four times in an outside hospital. He was transferred to the Peter Bent Brigham Hospital on April 16, 1959, because of the possibility of transplantation from an identical twin brother. On admission he was a pale, obviously ill young man whose blood pressure was 130/90 mm. Hg. There was a grade 1 systolic murmur at the left sternal border. Physical examination was otherwise within normal limits. Blood urea nitrogen was 61 mg. per cent, serum sodium 147 mEq./L., serum bicarbonate 24 mM/L., serum chloride 103 mEq./L., hematocrit 29 per cent. It was planned to perform a kidney graft from his identical twin brother. Two previous recipients of renal isografts with acute renal disease had contracted the disease in the grafted kidney. For this reason both of the patient's own kidneys were removed on the fifth hospital day, it having been previously ascertained that his identical twin would be a satisfactory kidney donor. Both diseased kidneys were large and showed evidence of subacute glomerulonephritis. The renoprival period lasted from the fifth to the fifteenth hospital day at which time a successful kidney isograft

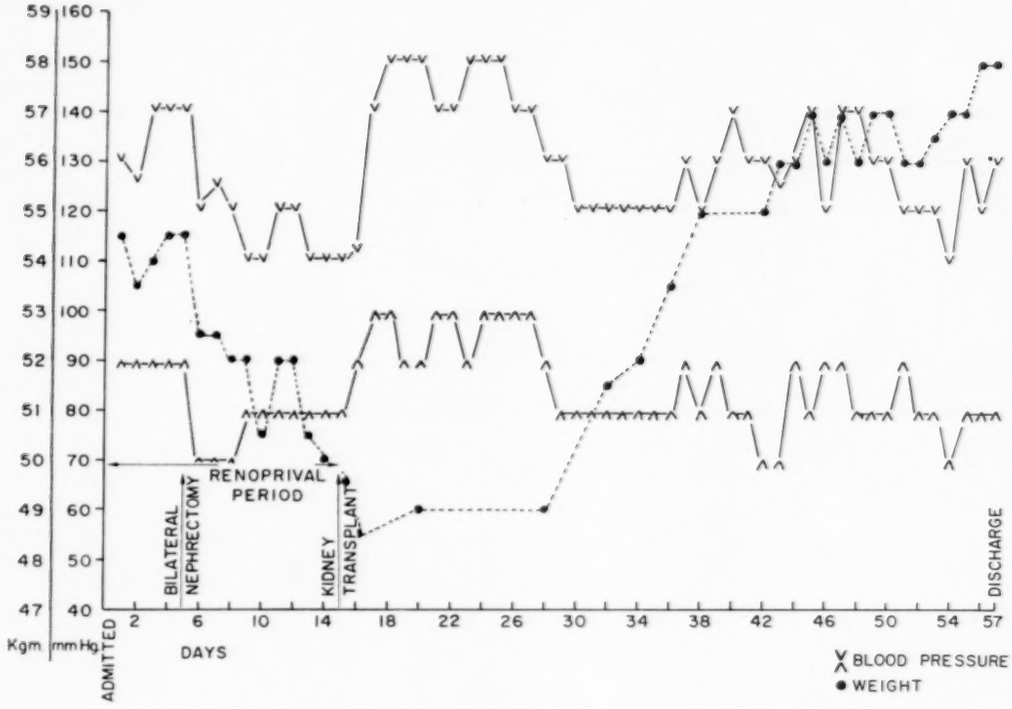


FIG. 5. Case IV.

was performed. During this time the patient was maintained on hypertonic glucose administered intravenously, sorbitol given orally and a multivitamin preparation. Weight, blood pressure and serum sodium concentrations are shown in Figure 3. Dialysis with the artificial kidney was performed on the twelfth hospital day because of increasing serum potassium concentration and corresponding electrocardiographic changes. In an effort further to depress possible antibody formation he was given a nitrogen mustard preparation intravenously (2.2 mg./kg. of nitrogen mustard prior to dialysis and 100 mg. of endoxan (cyclophosphamide) immediately after dialysis). A renal isograft was performed on April 30 at which time a biopsy specimen of an artery from the patient was taken, which showed minimal arteriosclerosis. Urine was elaborated from the transplanted kidney within eight hours and the blood pressure rose. (Fig. 5.) The urine volume gradually increased, renal function improved and the blood urea nitrogen was normal after the fifteenth operative day. The patient's convalescence thereafter was uneventful and the blood pressure returned to normal.

Comment: This patient entered the hospital with subacute glomerulonephritis and minimal hypertension. An elective bilateral nephrectomy was performed and for ten days, during which he had no renal tissue, the blood pressure remained normal. Body weight showed a decline consistent with normal hydration in a patient with inadequate caloric intake. A rise in blood pressure occurred with transplantation of a normal kidney from his identical twin brother. Although this kidney eventually functioned normally, it was ischemic from the period when its blood supply was occluded until it was reestablished in the recipient. During this period some parenchymal damage apparently occurred since there was an eight-hour period of virtual anuria, and normal renal function was not obtained for a period of two weeks. This evidence of temporary damage to the renal homograft is of interest in connection with the rise in blood pressure which occurred when it was transplanted.

COMMENTS

The data in these four patients completely devoid of renal tissue for periods of ten to sixty-two days indicate that in man, unlike the experimental animal [*16*], the absence of renal tissue alone is not of itself sufficient to cause hypertension. It has been pointed out that overhydration [*18*], sodium-containing electrolyte infusions [*23*], and a high protein intake [*28*] may produce hypertension in bilaterally nephrectomized dogs. A high protein intake may do this even in the absence of exogenous sodium excess [*17,24*].

In our patients there appeared to be no relationship between the presence or absence of hypertension and the serum sodium level, such as has been found in the animal experiments [*24*]. There did, however, appear to be some relationship of blood pressure levels to body weight, and this is consistent also with experimental data [*25*]. As in the experimental animal [*25*], the blood pressure in man falls following a dehydrating regimen once hypertension has become apparent. Nephrectomized animals may show an increase in body water even in the absence of hypertension [*24*]. This may also be true of the anuric human subject with renal tissue but with loss of excretory function [*21*]. Extracellular fluid volume as measured by the "inulin space" is consistently expanded in the anuric human subject [*26*]. It seems immaterial to argue that absence of hypertension reflects previous depletion of extracellular volume [*19*]. Clinical experience with subjects in acute renal failure of any sort indicates that the maintenance of body weight with an inadequate caloric intake and the appearance of hypertension precedes pulmonary edema or convulsions [*21*]. Under these circumstances what may be a normal extracellular fluid volume for a healthy person cannot be so construed for the patient with acute renal failure. Loss of lean tissue with inadequate caloric intake and increased metabolism requires a similar decrease in the volume of body fluid.

In our patients the diet was virtually protein-free except for one (Case III) whose diet contained less than 20 gm. of protein per day for short periods. The absence of hypertension might, perhaps, be related to this factor. The recent studies of Muirhead [*28*] suggest some correlation between hypertension and the degree of azotemia. Preliminary experiments in our own laboratory suggest that in rabbits loaded with urea following ureteral ligation, hypertension may develop [*29*]. No correlation, however, could be found between the concentration of blood urea nitrogen and the presence of hypertension in the present series of patients. Kolff and Page [*30*] also were unable to relate the level of the blood urea to the severity of the hypertension in dogs following bilateral nephrectomy.

Several other comments might be made in contrasting this experience in man with animal experiments. The point has been made that in dogs bilateral nephrectomy may result in hypertension whereas transplantation of both ureters into the vena cava does not. The inference is drawn that lack of renal tissue rather than lack of excretory function is the causative factor [*27*]. Our experience with man would not bear this out since the human subject with two damaged kidneys from which no urine is being elaborated behaves in a fashion similar to our renoprival patients, i.e., marked hypertension does not occur, except in the presence of overhydration.

On the other hand, it appears from our observations that the addition of an ischemic kidney to a normotensive patient without other renal tissue may provoke a rise in blood pressure. This transient hypertension disappears when the renal homograft has recovered from the ischemic episode (Case IV). It has been suggested [*5*] that "the abnormal constrictor activity of renoprival hypertensive plasma is due to an increase in circulating epinephrine-like material but that this humoral constrictor factor in renoprival rats acts only on arterioles sensitized in some way by the protracted arenal condition." In the one patient of this series in whom the study was carried out there was no increased vasomotor activity. In another case catecholamine levels seemed to be somewhat elevated but the validity of this colorimetric determination is questionable in the presence of marked uremia. Testosterone was administered routinely to three of our renoprival patients, and although this agent has been shown to cause a rapid elevation of the blood pressure in animals with decreased renal mass [*11*], this was not the case in our patients. No significant vascular lesions were present at autopsy in the three patients who died, although it has been reported in dogs that the renoprival state may give rise to vascular lesions [*27,28*].

As already pointed out, interpretation of the results of the relevant animal experiments is still controversial. Some authors [*18,23,25*] believe that renoprival hypertension does not occur in the absence of excess hydration. Others [*17,19*] believe that overhydration is not essential. This latter view has been most recently summarized by Muirhead [*31*] as follows: "These observations have been taken to indicate the existence of a native drive toward hypertension following renal ablation which is not necessarily dependent upon exogenous factors." Our experience with this limited series of human subjects leads us to disagree with the presence of "a native drive toward hypertension" in the

renoprival subject. In our patients maintained for long periods in a renoprival state without excess of salt, protein or water, hypertension did not occur except in the presence of overhydration or following the transplantation of a kidney with ischemic damage. We believe, therefore, that in man hypertension in the renoprival state, if it occurs at all, is dependent upon exogenous factors. The development of hypertension following the transplantation of a kidney with inadequate excretory function suggests that the presence of abnormal tissue is essential for the development of "renal hypertension" in man.

SUMMARY AND CONCLUSIONS

Four patients are described who were maintained without renal tissue for periods of ten to sixty-two days. Hypertension did not develop during the renoprival state on a low protein diet in the absence of excess hydration. When hypertension did appear as a result of excess hydration, dehydration brought the blood pressure to previous normal values.

In three of the four patients transplantation of an ischemic kidney was accompanied by a rise in blood pressure.

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On the Function of the Endocrine Glands in Myotonic Muscular Dystrophy*

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MYOTONIC muscular dystrophy (myotonia dystrophica, Steinert's disease) is a disease of unknown cause characterized by familial incidence, inheritance as a dominant trait with variable penetrance [*2*], myopathy involving chiefly the distal musculature of the extremities and cranial muscles, the myotonic phenomenon, cataracts and osseous lesions [*3,4*]. Three other features of myotonic muscular dystrophy that have aroused interest concern the function of the endocrine glands in this disorder. These are testicular atrophy, low basal metabolic rate and the frequent finding of subnormal values for urinary 17-ketosteroids [*5,6*]. Aside from their intrinsic interest as characteristics of myotonic dystrophy and not of other forms of muscular dystrophy [*5*], these manifestations have directed attention to the possible role of humoral factors in the pathogenesis of the disease.

Testicular atrophy is the most prominent glandular abnormality in myotonic muscular dystrophy [*5,6*]. The muscular weakness and poor muscle development of eunuchs have raised the possibility of a relationship between androgen deficiency and the muscle wasting which is characteristic of the disorder [*7*]. It is unlikely that such a relationship would be obligatory, in view of the fact that women have myotonic muscular dystrophy (there is no sexual predisposition) and in males atrophy of the muscles often precedes atrophy of the testes. Nevertheless, several attempts have been made to treat afflicted patients with testosterone [*7*]. The effects of adrogen therapy upon muscular function, measured by various mechanical indices, have been reported to be beneficial in two instances [*7*], equivocal or negative in the rest [*4,8,9*].

The common finding of a low basal metabolic rate has naturally suggested the possibility of reduced thyroid function [*4,8*]. This possibility is of special interest in view of the reported instances of the myotonic phenomenon occurring in myxedema [*10–12*], and allegedly disappearing after adequate thyroid replacement therapy [*10,12*]. The concept of hypothyroidism as an important mechanism of pathogenesis of myotonic dystrophy seems hard to sustain, since this association is very rare; overt hypothyroidism in a patient with myotonic muscular dystrophy has been reported only once [*23*], and more specific indices of thyroidal function, e.g., thyroidal I^{131} uptake [*6,13,14*], plasma half-life of administered I^{131} [*14*], response to thyrotropin [*6,14*], and serum precipitable iodine values [*13,14*] are generally normal.

The question of reduced adrenal cortical capacity has been raised by pathologic findings [*15–17*], by the observation of low urinary 17-ketosteroid values [*4,6,15,18*], by the reportedly inadequate response of plasma and urinary steroid levels to stimulation with adrenocorticotropin [*9,19*], and the frequent improvement of the myotonic phenomenon (but not of the muscular atrophy) following the administration of ACTH or adrenal cortical steroids to patients with the disease [*9,20–22*]. Whatever the implications of these findings may be, no instance of Addison's disease has yet been reported in association with myotonia dystrophica [*5*].

Disorders of other endocrine organs have also been implicated. "Minor abnormalities"

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have been described in the adenohypophysis studied at necropsy [*16*]. Diabetes mellitus has been said by some authors to occur with increased frequency [*3*], by others that there is no association between the two diseases [*23*]. The function of the parathyroids has been reported as normal [*4,8*].

The present study was undertaken in order to define more clearly the function of the endocrine glands in patients with myotonic muscular dystrophy. A critical review of the literature of the past twenty years has shown that certain of the conclusions cited have been based upon methods now considered outmoded or inadequate, that many studies have suffered from lack of sufficient controls (e.g., groups of patients with other chronic, wasting diseases), and that in some instances interpretation of acceptable data has been faulty. For example, in a recent textbook on inborn errors of metabolism it is stated that "most patients with myotonia fail to reproduce. Other endocrine disturbances include deficiencies of the thyroid, adrenal and pituitary glands" [*24*]. Similar statements appear in several textbooks of neurology and medicine. A careful study of the literature and appraisal of the data to be presented herein show, in fact, that most patients with the disease are fertile, and that indices of thyroid, adrenal cortical and pituitary activity almost always reveal normal function of these glands.

METHODS AND MATERIALS

Studies were made in seventeen patients with myotonic muscular dystrophy. There were nine men and eight women, ranging in age from twenty-eight to sixty-eight years. The muscular disease had been present by history for periods of one to twenty-seven years in these subjects. Diagnosis was established by the presence of varying degrees of muscular atrophy in the typical distal distribution, the myotonic response clinically, and, in all but one instance (patient H. F.),* electromyographic evidence of myotonia. Eight patients gave a family history of the disease, eleven had cataracts. Seven of the nine men had clinically apparent atrophy of the testes. Frontal baldness was present in all the men and in two of the eight women.

Thyroid. The basal metabolic rate was measured with a Benedict-Roth spirometer using the standards of DuBois. The twenty-four–hour thyroidal uptake of I^{131} was measured with a bismuth cathode Geiger tube (distance 15 cm., window opening 10 to 13 cm.) after oral doses of 8 to 15 μc. of the isotope. Serum protein-bound iodine was determined by the method of Barker, Humphrey and Soley [*25*],* serum butanol-extractable iodine by the method of Man, Kydd and Peters [*26*], and cholesterol by the technic of Schoenheimer and Sperry [*27*]. In four patients (M. G., J. L., M. U. and R. R.) microscopic examination of the thyroid was possible.

Adrenal Cortex. Urinary steroid values were measured in eleven patients. Twenty-four–hour urine specimens were refrigerated without preservative. 17-ketosteroid levels were determined by the method of Holtorff and Koch [*28*] or that of Sobel et al. [*29*]. Urine 17-hydroxycorticosteroids were measured by the technic of Silber and Porter [*30*] after β-glucuronidase hydrolysis at pH 5 and 37°C. for forty-eight hours. Urinary 17-ketogenic steroids were determined by Norymberski's method as modified by Sobel and co-workers [*29*].

The response of plasma 17-hydroxycorticosteroids to adrenocorticotropin was assessed in twelve patients. Adrenocorticotropin (25 I.U.) was given intravenously over a four-hour period [*31*] in an effort to achieve maximal stimulation, since intramuscular administration of ACTH has been reported to elicit minimal or subnormal responses of the adrenal cortex in this disease [*9,19*]. Plasma steroids were determined by the Silber-Porter technic [*30*] as modified by Peterson [*32*] and in this laboratory [*33*].

In two patients (W. K. and H. A.) the rate of clearance of cortisol from plasma was determined. Cortisol hemisuccinate,† 100 mg., was given intravenously, and serial blood specimens were withdrawn at twenty, forty, sixty, 120 and 240 minutes in accordance with a schedule employed earlier in a study of cortisol clearance in normal and pregnant subjects and in patients with Laënnec's cirrhosis [*34*]. The cortisol clearance rate in myotonia was investigated in order to evaluate the plasma steroid response to ACTH. As Eik-Nes et al. have pointed out, the rate of cortisol rise after ACTH stimulation (now a standard method of assessing adrenal cortical function) depends upon at least two variables: (1) the rate of increase of adrenal cortical secretion of cortisol, and (2) the rate of cortisol disappearance from plasma [*35*]. If cortisol leaves the plasma at an abnormally slow rate, a subnormal adrenal cortical response to ACTH may appear to be normal [*34,36*], or a normal response may appear to be exaggerated [*34,37*]. From these data, the cortisol secretion rate was calculated [*37*] (see Results).

One subject (K. D.) was observed during the third

* This woman and her relatives have a myopathy which, in its distal and cranial distribution, is identical with that of myotonic muscular dystrophy. Ophthalmoplegia is also part of the syndrome, but myotonia, as measured clinically or by electromyography, has not been demonstrated. This syndrome will be described in detail elsewhere.

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† Supplied through the courtesy of The Upjohn Company, Kalamazoo, Michigan.

TABLE I

INDICES OF THYROID FUNCTION IN PATIENTS WITH MYOTONIA DYSTROPHICA

Patient	Sex and Age (yr.)	Palpation of Thyroid Gland	Basal Metabolic Rate (%)	I^{131} (24-Hour) Uptake (%)	Serum Protein-Bound Iodine (μg. %)	Serum Butanol-Extractable Iodine (μg. %)	Serum Cholesterol (mg. %)	Thyroid Histology (necropsy)
W. K.	M,29	Normal	−26	29	5.3	3.9	187	...
H. A.	M,30	Normal	−32	15	5.6	...	...	...
A. S.	M,40	Normal	−22	10*	6.8	4.8, 6.0	151	...
M. G.	M,44	Normal	−37	22	...	...	173	Normal
J. Li.	M,47	Normal	−2	...	4.4	...	187	...
C. G.	M,63	Normal	−8, −16	31	7.6	...	253	...
J. L.	M,52	Mass, right lobe	+13	39†	...	...	165	Adenoma
M. U.	M,58	...	−18, −23	32	...	...	141, 214	Normal
R. R.	M,60	Normal	...	...	...	...	...	Normal
M. H.	F,66	Normal	−1, −6	...	6.6	...	194	...
H. F.	F,68	Normal	...	42	6.5	...	243	...
R. M.	F,53	Normal	−35	17	5.9	...	260	...
A. E.	F,67	Non-toxic nodular gland	−26	38	5.7	...	194	...
K. D.	F,28	Normal	−4	...	5.9, 8.3‡	...	208	...
V. V.	F,49	Normal	−26	19	5.8	...	284	...
A. B.	F,47	Normal	−25	7§	...	...	328	...
M. T.	F,40	Normal	−26	Normal	...	...	...	...
Normal values		−10 to +15		15–50	4–8	3–7	150–250	...

* Determination made after ingestion of Lugol's solution.

† Value fell to 18 per cent after a course of triiodothyronine therapy (normal response [*44*]).

‡ Expected rise during pregnancy [*38*].

§ Determination made after ingestion of thyroid extract.

trimester of pregnancy. The urinary and plasma steroid responses to ACTH were determined to learn whether or not the apparent exaggerations in these indices occurring in normal pregnancy could be shown in a woman with myotonia dystrophica [*38*].

Serum sodium was measured by flame photometry in eleven subjects.

Gonads. The function of the testis was assessed by the history of libido and fertility, measurement of height (upper versus lower segment) and arm span, distribution of body hair and, in seven of the nine men, histologic study of the testis. In order to determine whether or not testicular failure was of pituitary origin, urinary gonadotropin excretion was assayed by the method of DeKanski [*39*], and in four instances by the method of Albert as well [*40*].*

Ovarian function was estimated by the history of menstrual cycles and of fertility, by physical examination of secondary sex characters and, in two instances, by histologic examination of the ovaries.

Pancreatic Islets, Parathyroids. Blood glucose was measured by the method of Folin and Wu [*41*] or by an Autoanalyzer® (Technicon Corporation, Chauncey, New York). Serum calcium was determined by the method of Kramer and Tisdall as modified by Clark and Collip [*42*], and serum phosphate by the Fiske-Subbarow method [*43*]; both measurements were made by means of the Autoanalyzer over the past two and a half years.

* These four determinations were carried out by Dr. Alexander Albert of the Mayo Clinic.

RESULTS

Thyroid. Data concerning thyroid function are summarized in Table I. Two subjects (J. L. and A. E.) had palpable goiters; one (J. L.) was shown to be a non-toxic nodular goiter when removed surgically. No patient had clinical evidence of thyrotoxicosis or myxedema. (One patient, A. B., was a forty-seven year old woman who had been suspected of having hypothyroidism by her previous physician and had received up to 3 gr. a day of desiccated thyroid, without apparent clinical change. When she was first seen in this clinic her basal metabolic rate was minus 25 per cent despite thyroid replacement therapy, I^{131} uptake was 7 per cent presumably as a result of suppression by exogenous thyroid,

TABLE II

INDICES OF ADRENAL FUNCTION IN PATIENTS WITH MYOTONIA DYSTROPHICA

Patient	Sex and Age (yr.)	Duration of Muscle Disease (yr.)	Serum Na^+ (mEq./L.)	Urinary 17-Keto-steroids (mg./24 hr.)	Urinary 17-Hydroxy-cortico-steroids (mg./24 hr.)	Urinary 17-Keto-genic Steroids (mg./24 hr.)	Plasma 17-Hydroxycorticosteroids (μg. %)	
							Before ACTH	After ACTH
W. K.	M,29	5	...	6.0	...	12.5	12	35
H. A.	M,30	1+	141	1.9	3.8	4.6	25	48
A. S.*	M,40	13	138	2.9, 3.7	...	8.8, 5.5	13	27
M. G.	M,44	10	142	1.8	...	...	...	...
J. Li.	M,47	2	147	4.5, 5.6	3.5	10.0, 10.3	28	47
C. G.	M,63	23	142	4.8	6.6	14.0	24	46
J. L.	M,52	1	Normal	...	...	...	18	37
M. U.	M,58	25	...	...	...	...	...	...
R. R.	M,60	14	...	...	...	...	...	...
M. H.	F,66	11	...	...	...	...	16	49
H. F.	F,68	16	146	1.2	1.9	6.5	22	51
R. M.	F,53	16	...	3.1, 9.6	...	9.9	29	41
A. E.	F,67	20	142	1.8	...	5.1	21	44
K. D.†	F,28	1	142	7.0	...	9.5	27	70
V. V.	F,49	21	140	14.9	...	16.2	11	57
A. B.	F,47	6	144	...	...	...	...	...
M. T.	F,40	27	...	...	...	...	...	...
Normal values			...	...	5–20	3–9	5–20	4–23	35–55

* Seminiferous tubule dysgenesis with positive nuclear chromatin [*46*].

† Study carried out in the third trimester of pregnancy. 17-ketosteroids and 17-ketogenic steroids values rose after ATCH administration to 17.1 and 24.2, respectively.

and the serum cholesterol was 328 mg. per cent. The diagnosis of hypothyroidism seemed questionable at best but the patient's lack of cooperation precluded complete evaluation.)

The basal metabolic rate was measured in fifteen patients and was distinctly low in eleven, and normal in four (J. Li., J. L., M. H. and K. D.). K. D. was in the third trimester of pregnancy when the measurement was made. I^{131} uptake by the thyroid was normal in eleven of thirteen patients. The low values in two (A. S., 10 per cent and A. B., 7 per cent) can be attributed to prolonged ingestion of Lugol's solution and desiccated thyroid, respectively. One patient (J. L.) showed normal suppression of I^{131} uptake from 39 to 18 per cent, after eight days of triiodothyronine ingestion [*44*]. Serum protein-bound or butanol-extractable iodine, determined in eleven patients, was normal in each, and showed the expected increase during pregnancy (K. D.). Serum cholesterol levels were normal in all but one patient (A. B.) (*vide supra*).

Histologic study of the three glands studied at necropsy (M. G., M. U. and R. R.) showed normal morphology.

In summary, data pertaining to thyroid function again reveal the characteristic hypometabolism of myotonia dystrophica, and confirm earlier findings which tend to exclude hypothyroidism [*6,13,14,45*].

Adrenal Cortex. Table II summarizes the data concerning adrenal cortical function. No patient had typical Addisonian pigmentation. The serum sodium was normal in all eleven patients in whom it was measured. Urinary 17-ketosteroids, measured in eleven patients, were low in eight; 17-hydroxycorticosteroids, measured in four subjects, were normal in three, low in one; urine 17-ketogenic steroids, measured in ten patients, were normal in all but one (H. A.). The response of plasma cortisol levels to intravenous ACTH was normal in all but two (K. D. and A. S.) of the twelve patients studied. The apparently exaggerated response shown by patient K. D. is typical of that exhibited by

normal pregnant women tested with ACTH in the third trimester of pregnancy [*31*]. Only one subject (A. S.) responded subnormally, a forty year old man with seminiferous tubule dysgenesis (Klinefelter's syndrome), gynecomastia and positive (female) nuclear chromatin [*46*]. It is of interest that his urine 17-ketosteroid value was 16.1 mg. per day (normal) when determined eight years before the current low values of 2.9 and 3.7 mg. per day were found.

Two subjects (W. K. and H. A.) with normal and low urinary 17-ketosteroid levels, respectively, showed normal rates of removal from plasma of infused cortisol. In both patients the plasma half-time of the administered steroid was eighty minutes (normal) [*47*]. The normal disposal rate of cortisol suggests that the normal cortisol rise after the administration of ACTH was not an artifact resulting from a delay in disappearance of the steroid from plasma. Calculation of the secretion rate of cortisol by the formula of Samuels et al. [*37*]* revealed basal secretion rates of 45 mg. cortisol per day (H. A.) and 37 mg. per day (W. K.). With maximal ACTH stimulation the calculated cortisol secretion rates rose to 214 mg. per day (H. A.) and 266 mg. per day (W. K.). These figures are in the range reported for normal subjects by Samuels et al. [*37*]. (Average cortisol secretion rate, 37 mg. per day without ACTH; 260 mg. per day after maximal ACTH stimulation. The figures are higher than those given for normal subjects by Peterson and Wyngaarden [*48*] who estimated turnover rates by isotopic technics and found the basal cortisol turnover rate to be on the average 22 mg. per day; after the administration of ACTH it rose to 116 to 154 mg. per day.) Although obtained by indirect means, estimates of secretion rate of cortisol in these two patients are taken as evidence in support of a relatively normal production of the principal adrenal cortical glucocorticoid, cortisol.

The histology of the adrenal was essentially normal in the three patients who came to necropsy (M. G., M. U. and R. R.).

In summary, the balance of the data appear to support the conclusion that adrenal cortical function is normal in this group of patients with myotonia dystrophica, with the possible exception of one subject (A. S.).

Gonads. Table III summarizes the data relating to gonadal function.

Testis. In seven of the nine men the testes were small; the testes of the other two were described as normal in size and consistency. The atrophied testes were all soft to palpation, except for those of one patient (A. S.) (chromatin-positive) in whom they were firm and irregular. This was the only subject who had eunuchoid proportions and gynecomastia. (This patient is assumed to represent a fortuitous association of true Klinefelter's syndrome and myotonic dystrophy [*46,49*].) Five of the six married men were fertile. A biopsy specimen of the testis in seven patients showed disorganization of spermatogenesis and varying degrees of tubular fibrosis. (The detailed morphology of the testis in this disorder is the subject of a separate report [*49*].) Only one (J. L.) of the six men tested had an elevated titer of urinary gonadotropin (positive at 80 mouse units per twenty-four hours). Gonadotropin excretion was normal in three, undetectable in two subjects.*

Ovary. The eight women showed generally normal secondary sex characters. Six had had full term pregnancies. One (A. E.) had histologically normal ovaries; another (R. M.) had an "orange-sized" cyst of the right ovary.

Anterior Pituitary, Parathyroids, Pancreatic Islets. Normal function of the anterior pituitary was

* The cortisol secretion rate, with and without ACTH stimulation, was calculated using the assumption that a constantly increasing amount of cortisol is being added to the plasma pool per unit time, and the excess is removed at a rate proportional to plasma concentration [*37*]. Where C = concentration of plasma 17-hydroxycorticosteroids in μg. per 100 ml., t = time, k = slope of cortisol removal curve and k′ = constant rate of cortisol input,

$$\text{Input:} \quad \frac{dC}{dt} = k'$$

$$\text{Removal} \quad \frac{dC}{dt} = kC, \text{ then}$$

$$\text{Composite change:} \quad \frac{dC}{dt} = k' - kC$$

Integrated between C_o and C_x (concentration at zero time and concentration at any arbitrary time, here four hours):

$$\ln \frac{\frac{k'}{k} - C_o}{\frac{k'}{k} - C_x} = kt$$

* By the DeKanski method [*39*], gonadotropin is often undetectable even in apparently normal subjects in this laboratory. Two patients (H. A. and A. S., Table III) had no measurable titers by the Albert method [*40*]; the concentrations in two others (W. K. and J. L.) were essentially the same by the two technics.

TABLE III

INDICES OF GONADAL FUNCTION IN PATIENTS WITH MYOTONIA DYSTROPHICA

Patient	Sex and Age (yr.)	Duration of Muscle Disease (yr.)	Eunuchoid Proportions	Genitalia	Fertility	Biopsy	Nuclear Chromatin	Urinary Gonadotropin (mouse units/24 hr.)
W. K.	M,29	5	No	Testes soft, small	Single	Tubular fibrosis	Negative	+5, −50
H. A.	M,30	1+	No	Testes soft, small	Single	...	Negative	−10, −40
A. S.*	M,40	13	Yes	Testes hard, atrophic	No	Germinal aplasia, tubules absent	Positive	−10, −80
M. G.	M,44	10	No	Normal	Yes	Tubular atrophy	Negative	...
J. Li.	M,47	2	No	Testes soft, small	No†	Tubular fibrosis	Negative	+10, −40
C. G.	M,63	23	No	Testes soft, small	Yes	Tubular fibrosis but some spermatogenesis	Negative	+10, −40
J. L.	M,52	1	No	Testes small	Yes	...	Negative	+10, +80
M. U.	M,58	25	No	Testes atrophic	Yes	Tubular fibrosis	Negative	...
R. R.	M,60	14	No	Normal	Yes	Tubular fibrosis	Negative	...
M. H.	F,66	11	...	...	No	...	...	−40
H. F.	F,68	16	...	Normal	Yes	...	...	+40
R. M.	F,53	16	...	Normal	Yes	Ovarian cyst	...	+10, −80
A. E.	F,67	20	...	...	Yes	Ovaries normal	...	+40
K. D.‡	F,28	1	...	Normal	Yes	...	...	...
V. V.	F,49	21	...	Normal	Yes	...	...	−10, −50
A. B.	F,47	6	...	...	No	...	...	...
M. T.	F,40	27	...	Normal	Yes	...	...	...
Normal values		...	...	...	...	...	...	5–20

* Seminiferous tubule dysgenesis with gynecomastia and positive nuclear chromatin [*46*].

† Married two times.

‡ Patient observed in the third trimester of pregnancy.

inferred from the apparently normal function of the adrenal cortex and thyroid and the presence of detectable gonadotropin in seven of eleven patients. Measurements of serum calcium and phosphorus in six patients gave normal values in all. The blood glucose determined in twelve subjects, showed that two (C. G. and M. U.) had frank diabetes mellitus, requiring control either by diet or an orally administered hypoglycemic agent, and two (R. R., A. E.) had elevated postprandial levels of blood glucose without diabetic symptoms and without glycosuria.

Endocrine Studies Reported in the Literature. Table IV is a synopsis of endocrine studies in patients with myotonic muscular dystrophy made over the past twenty years. It will be observed that urinary 17-ketosteroid values are usually below the lower limit of normal, but that urinary corticosteroids, measured by seven different methods, are normal as a rule. The response of urinary and plasma steroid levels to ACTH stimulation tends to be subnormal when ACTH is given intramuscularly [*9,19*], but normal when the hormone is given by the intravenous route [*6*]. In about 60 per cent of cases the basal metabolic rate is low. Uptake of radioactive iodine and serum precipitable iodine have been generally normal. There are isolated instances of myxedema [*23*] and thyrotoxicosis [*50*]. Testicular atrophy has been found in about 80 per cent of the male patients. Tubular fibrosis is the usual histologic finding. Normal or reduced titers of urinary gonadotropin are the rule; the titers were elevated in only 16 per cent of patients studied. Despite the frequently repeated statement that there is a high incidence of menstrual irregularities and of sterility in women with myotonic dystrophy, these abnormalities occurred in only 15 to 20 per cent of the reported patients, an incidence not ap-

preciably different from that found in chronic, debilitating diseases generally.

COMMENTS

The data presented indicate normal adrenal cortical, thyroid and ovarian function in patients with myotonic muscular dystrophy. These findings are in general agreement with those of several recent studies [*6,14,45,51*], not with others [*4,9*]. Testicular atrophy has been confirmed in about 80 per cent of males, but many of these had been fertile at one time. There appears to be an unexpectedly high incidence of diabetes mellitus. In sum, since most endocrine functions are normal, there seems to be no basis for the notion that endocrine deficiencies play an important role in the pathogenesis of the muscular lesion [*14*].

The data of the present study, together with those obtained from the literature do not, on critical analysis, bear out a number of commonly held ideas about glandular function in myotonia dystrophica. There are several reasons for this. For example, it has been assumed by some that adrenal cortical reserve is low because a few patients have responded subnormally to *intramuscularly* administered ACTH [*9,19*]. The normal responses in the patients of this and other [*6,14,45*] series to *intravenously* administered ACTH suggest that the intramuscular route of administration failed to provide a maximal stimulus to the adrenal cortex. Considerable doubt must be cast upon the specificity of low urinary steroid excretion values in myotonic dystrophy by the fact that these values are often low in a great variety of chronic, wasting diseases [*52–54*]. In respect to the inference of testicular (i.e., Leydig cell) insufficiency from the consistently low urinary 17-ketosteroid values [*4,15,55*], it is now known that orchiectomy does not bring about a major reduction in urinary ketosteroids, which may fall somewhat but still remain well within the limits of normal [*56,57*]. Such evidence suggests that the testicular secretions contribute no more than 15 to 20 per cent of the daily hormone, which is ultimately metabolized to "crude" 17-ketosteroids (i.e., 17-ketosteroids measured by the usual clinical methods). Although urinary ketosteroids have been reported to rise after the administration of chorionic gonadotropin [*6,58,59*], they also respond to the administration of ACTH [*6,59*] in men with this disease. We submit that the low values, characteristic of numerous chronic diseases [*52–54*], by no means specific for myotonic muscular dystrophy, and not ascribable to hyposecretion of testosterone by Leydig cells, may well be due to a deficiency of the adrenal cortical androgen, whatever that may be.

TABLE IV
INDICES OF ENDOCRINE FUNCTION IN PATIENTS WITH MYOTONIC MUSCULAR DYSTROPHY, COMPILED FROM THE LITERATURE, 1940–1960

Index	No. of Patients or Measurements	Comment
Adrenal cortex		
Urinary 17-ketosteroids	121	73% subnormal 27% normal or low normal
Urinary "corticoids"	25	84% normal
Thyroid		
Basal metabolic rate	69	61% subnormal (−10%)
I^{131} uptake	36	All but one normal
Protein-bound iodine	19	All but one normal
Serum cholesterol	74	All but three normal
Gonads		
Genetic sex	39 males	All but one male (chromatin negative [*46*])
	13 females	100% female (chromatin positive)
Fertility	26 males	85% fertile
	21 females	80% fertile
Urinary gonadotropin*	86	16% elevated 57% normal 27% subnormal
Status of testes	129	81% atrophy
Pancreatic islets†	40	18% overt diabetes mellitus
Parathyroids	43	All but one normal

* Gonadotropin levels were reported for seventy-four men and twelve women. Only one woman had a high titer (age fifty-one, postmenopausal); among the men, increasing age did not correlate with gonadotropin titer, one man with a high value was fifty-six years old, all the rest were younger.

† The 18 per cent figure for diabetes mellitus does not include patients who exhibited simply a high blood sugar level on casual determination (*cf.* effect of inanition).

It would thus appear that, other than testicular atrophy, usually occurring in early middle life, endocrine abnormalities are not an important feature of myotonic muscular dystrophy [*6,14,45,51*]. The relatively high incidence of diabetes mellitus is perhaps less important as an endocrine concomitant of myotonia than as a genetic disorder in its own right. Further statistical correlation of myotonic dystrophy with diabetes mellitus and with chromosomal abnormalities (e.g., patient A. S., a chromatin-positive phenotypic male) may be expected to yield genetic information of interest.

In addition to the unexplained low values for urinary 17-ketosteroids with normal corticoids, the low basal metabolic rate in the face of overwhelming evidence in support of normal thyroid function also remains a mystery. The low basal metabolic rate has been related to reduced lean

body mass [*5*]. Such an explanation has three faults. First, loss of muscle mass is often minimal even in the presence of weakness, at a time when the basal metabolic rate is subnormal. Second, creatinuria, which is attributed to reduced muscle mass in other forms of muscular dystrophy, is an uncommon feature of myotonic dystrophy [*60*]. Third, patients with other dystrophies, characterized by creatinuria and probably reduction in lean body mass, do not show low metabolic rates [*5,60*]. There is no satisfactory explanation, as yet, for the hypometabolism, and the subject remains one for future investigation.

SUMMARY AND CONCLUSIONS

A study is presented of endocrine function in seventeen patients with myotonic muscular dystrophy, nine men and eight women, aged twenty-eight to sixty-eight years.

Although the basal metabolic rate was low in eleven of fifteen subjects, I^{131} uptake by the thyroid was normal in eleven of thirteen, and the serum protein-bound iodine was normal in all eleven patients studied. The low basal metabolic rate, typical of this and not of other forms of muscular dystrophy, remains unexplained.

Adrenal cortical function was normal. Of eleven patients investigated all had normal serum sodium concentrations; urinary 17-ketogenic steroids were normal in all but one of ten patients; the response of plasma cortisol to intravenously administered ACTH was normal in eleven of twelve; and the calculated secretion rate of cortisol was normal in both of two patients studied.

Ovarian function was generally adequate. Secondary sex characters were normal, and six of eight women had one or more normal full term pregnancies with spontaneous delivery.

Primary atrophy of the testis, which occurs sooner or later in about 80 per cent of men with the disease, was present in seven of nine men. The nuclear chromatin pattern, when studied, corresponded to the phenotype in all but one instance. Five of the six married men had been fertile. Four of six had detectable gonadotropin in the urine; only one of the four had an elevated titer.

There is a moderately high incidence (18 per cent) of diabetes mellitus, itself a familial disorder.

A critical review of the literature of the last twenty years, taken together with the data of the present study, affords no basis for allegations of a high frequency of adrenal cortical, thyroidal, ovarian and pituitary deficiencies in myotonic muscular dystrophy. The facts seem to indicate that the function of these glands is normal, so far as one can determine with the methods now available.

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Case Reports

Angiokeratoma Corporis Diffusum Universale (Fabry)*

Presenting as Suspected Myocardial Infarction and Pulmonary Infarcts

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As reports of the rare disease, angiokeratoma corporis diffusum universale, accumulate, a more clearly defined clinical syndrome becomes apparent. The following case illustrates an interesting manner in which the disease may present itself, with a rather striking cardiopulmonary disorder. Following more careful inquiry the other varied features became evident. It is noteworthy that an apparently benign type skin eruption, from which the syndrome is named, heralds the more extensive underlying systemic vascular disease.

CASE REPORT

A twenty-nine year old white man was admitted to the Travis Air Force Base Hospital on April 7, 1959, because of hemoptysis and severe chest pain. He had been in good health until about eight months before admission when he noted the onset of striking polyuria, 2 to 4 quarts per day. At this time he was stationed with the U. S. Army in Korea. Six months before admission the patient had a gradual onset of weight loss, from 208 pounds to his present weight of 155 pounds. Four months prior to admission he experienced shortness of breath on walking about one block. This became more severe subsequently and markedly limited his activities. It was followed by an episode of self-limited diarrhea. Three months prior to admission the patient was seen at another medical facility complaining of fever and chills for a period of five days. This was considered a possible infection of the upper respiratory tract and subsided after treatment.

On February 12, 1959, two months before admission, the patient had gradual onset of constant substernal and xyphoid pains in the chest associated with nausea and vomiting. The pain progressed and radiated into both arms and elbows along the ulnar distribution. It was aggravated by deep breathing and reclining, and relieved somewhat by sitting up. The patient was hospitalized. He was found to be afebrile, no friction rubs were heard, electrocardiograms demonstrated S-T and T wave changes over an eight-day period, as shown in Figure 1. The pain subsided spontaneously in two days. Nitroglycerin therapy provided no relief. On February 15, 1959, the day of admission, the white blood cell count was 16,000/cu. mm.; this dropped to 5,000 cells/cu. mm. on the following day and subsequently ranged between 9,600 and 12,500 cells/cu. mm. The differential count showed 60 to 80 per cent neutrophils. The erythrocyte sedimentation rate varied between 10 and 28 mm. (Wintrobe). Initial SGOT level, on February 19, was 32 units; the following day it was 23 units.

On March 2, 1959, the patient was ambulated and felt much improved; however, he still had intermittent dull aching pains in the anterior region of the chest. On March 9, the patient had an episode of nausea and vomited about 1 teaspoonful of bright red blood. The following day he coughed up small amounts of bright red blood. He was transferred subsequently to another hospital on March 16, 1959, where the clinical data including electrocardiograms were reviewed, and the diagnosis of myocardial infarction was made. It was their opinion that the hemoptysis could best be explained by pulmonary infarctions, although a roentgenogram of the chest and physical examination of the chest remained within normal limits. He continued to have intermittent, hemoptysis consisting of several teaspoonfuls of bright red blood per day and intermittent, migratory, severe, pleuritic chest pains. Because of the diagnosis of pulmonary infarction, Dicumarol® therapy was instituted. About this time the patient recalled, in retrospect, noticing bright red angiomatous lesions on the anterior portion

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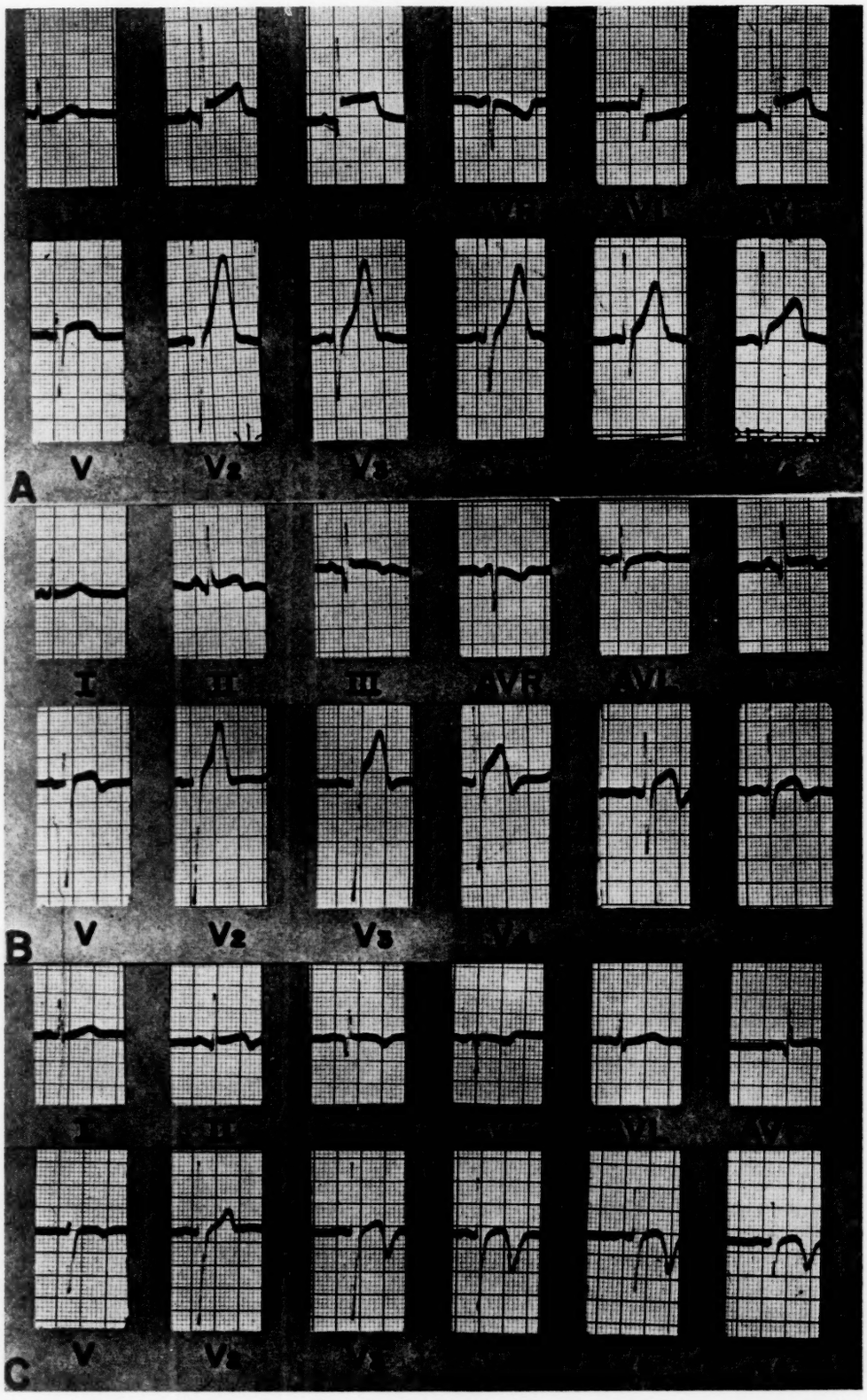


FIG. 1. Electrocardiograms taken during initial hospitalization show a pattern of acute myocardial damage mostly to the posterolateral wall, with rapid evolution. A, during morning of February 16, 1959. B, afternoon of February 16, 1959. C, on February 24, 1959.

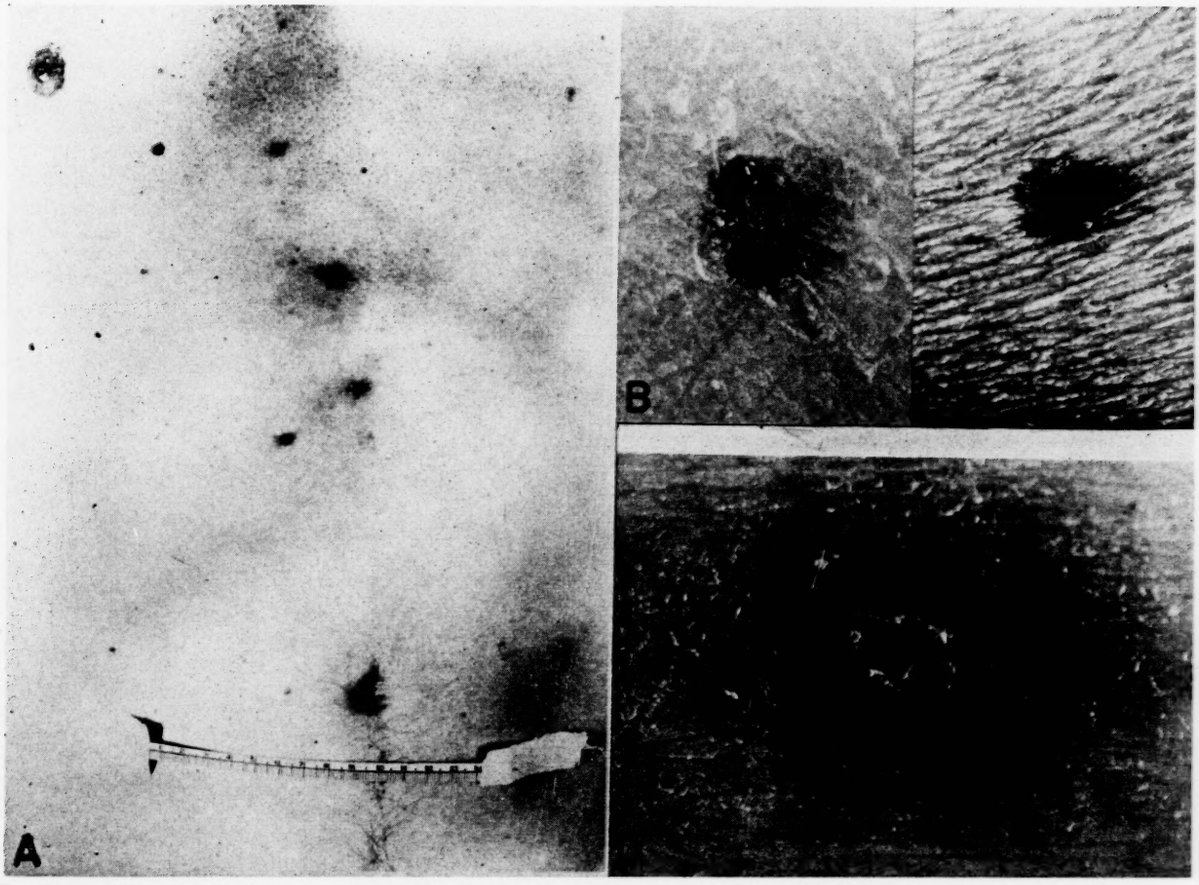


FIG. 2. A, diffuse bright red angiomas scattered over the anterior part of the trunk. A few older, larger, hyperkeratotic, brown pigmented lesions are also present. B, a close up view of an acute angioma which erupted on the right foot during hospitalization and evolved through stages shown in C and D. C, a bright red angioma with a fine, hyperkeratotic plaque visible along the lower margin. D, the oldest type angioma which has become larger, diffusely brown in color and hyperkeratotic in the center.

of the chest and on the abdominal area. He stated that at the time of the initial hemoptysis he passed some bright red blood in his stool, but no notes are made of this in the referring hospital records. Following the initiation of Dicumarol therapy no improvement occurred. Migratory pleuritic chest pains and intermittent, bright red hemoptysis continued unchanged. Because the patient was located in an overseas facility he was transferred to the Zone of the Interior for further evaluation.

The patient was born in Alabama. He completed high school and was an automobile mechanic until he entered the service. He admitted to heavy alcoholic intake from 1951 to 1953 but stated he had had none since. He smoked one package of cigarettes per day. There was no history of drug sensitivities. He had the usual childhood illnesses without complications and his only other hospitalization was in 1952 for streptococcal pharyngitis, which quickly cleared with penicillin therapy. His father, aged sixty, his mother, aged fifty-eight, and his sister, aged twenty-three, were well. Three paternal uncles apparently died from heart attacks at about forty years of age.

Physical examination revealed a chronically ill appearing, thin, gaunt white man in moderately acute distress complaining of intermittent, sharp, anterior chest pains. Blood pressure was 120/80 mm. Hg; pulse, 72; respirations, 14; temperature, 98.8°F.; no parodoxical pulse was demonstrated. Examination of the skin showed multiple, small, bright red, angiomatous lesions over the anterior and posterior trunk region with a few scattered similar lesions on the arms and feet. Over the anterior part of the chest were multiple 0.5 to 1 cm. maculopapular, slightly raised, pigmented, hyperkerototic lesions which the patient stated had initially been similar to the bright red, angiomatous lesions, but had progressed to this type lesion over a period of several weeks. The bright red angiomas were slightly raised, and over some a fine, slightly roughened, hyperkeratonic plaque could be seen and palpated. (Fig. 2.) The examination of the eyes, ears, nose and throat was within normal limits. Examination of the heart disclosed a regular rhythm, no murmurs and no friction rubs. The chest was symmetric with normal respiratory movements. The lungs were clear. Examination of the abdomen failed

TABLE I

PULMONARY FUNCTION STUDIES IN A PATIENT WITH ANGIOKERATOMA CORPORIS DIFFUSUM UNIVERSALE

Studies	Patient's Values	Normal Range
Lung Volumes (BTPS)		
Residual volume (RV) (ml.)	2,877	1,100–1,700
Functional residual capacity (ml.)	3,877	1,800–3,000
Ventilation (BTPS)		
Tidal volume (ml.)	424	400–600
Frequency (respirations/min.)	18.6	12–16
Minute volume (ml./min.)	7,240	5,500–7,000
Respiratory dead space (physiologic) (ml.)	173	150
Alveolar ventilation (ml./min.)	4,670	4,200
Distribution		
Dead space ventilation/alveolar ventilation ratio (%)	41	30
Alveolar Gas		
Oxygen partial pressure (mm. Hg)	112	104
CO_2 partial pressure (mm. Hg)	33.5	40
Diffusion and Gas Exchange		
O_2 consumption (STPD) (ml./min.)	224	250
CO_2 output (STPD) (ml./min.)	203	200
Respiratory exchange ratio (CO_2 output/O_2 uptake)	0.91	0.80
Arterial Blood Studies		
O_2 saturation (room air) (%)	94.3	97
O_2 tension (mm. Hg)	75	95
O_2 saturation (13% O_2) (%)	72.4	75
CO_2 tension (mm. Hg)	33.5	40
Alveolar-arterial PO_2 difference (room air) (mm. Hg)	37	9
Alveolar-arterial PO_2 difference (12–14% O_2) (mm. Hg)	16.6	10
pH, arterial	7.49	7.40

to disclose any masses or tenderness. There was no lymphadenopathy. Neurologic examination was within normal limits. Rectal examination revealed no abnormalities. Examination of the extremities disclosed no peripheral edema. The soles of the feet were markedly hyperkeratotic; this, according to the patient, had been present for several months.

Laboratory data: The hematocrit was 46 per cent; white blood count, 8,250 cells/cu. mm. with 63 per cent neutrophils, 37 per cent lymphocytes; erythrocyte sedimentation rate, 12 mm. (Wintrobe); peripheral blood smear, adequate platelets and normal red cell morphology; platelet count, 102,000/cu. mm.; results of the tourniquet and cardiolipin microflocculation tests were negative; antistreptolysin O titer, 100 Todd units; SGOT, 12, 23 and 24 units, respectively, on the first three days of hospitalization. Urinalysis showed a specific gravity of 1.007; albumin and sugar, negative; microscopic, 5 to 6 white blood cells/high power field. Initial prothrombin time, thirty-two seconds (fifteen seconds after discontinuation of Dicumarol therapy); heterophil antibody titer, 1:7; C-reactive protein, 1 plus; Ivy bleeding time, two minutes; Lee-White clotting time, four minutes; clot retraction, good in four hours. The albumin was 4.2 gm. per cent; globulin, 3 gm. per cent; blood urea nitrogen, 12 mg. per cent; creatinine, 1.6, 2.3, 1.4 and 1 mg. per cent, respectively; protein-bound iodine, 4.8 μg. per cent; sodium, 148 mEq./L.; potassium, 4.6 mEq./L.; chloride, 106 mEq./L.; CO_2, 24 mEq./L.; bilirubin, 0.08 mg. per cent, direct and 0.16 mg. per cent, total; the result of the thymol turbidity test was 0.66 units and of the cephalin flocculation test, 1 plus in forty-eight hours; bromsulfalein retention, 1.5 per cent in forty-five minutes; cholesterol, 165 and 162 mg. per cent; esterified cholesterol, 118 and 114 mg. per cent; total lipid, 581 and 563 mg. per cent; phospholipid, 150 and 173 mg. per cent. Creatinine clearance was 132 ml. per minute. Twenty-four hour deprivation of water produced a total output of urine of 3,000 ml. with a specific gravity of 1.007. This was immediately followed by a pitressin concentration test, the patient receiving 2 units of pitressin subcutaneously, every 30 minutes for four doses. Urine specific gravity and volume was obtained initially and thirty minutes after each dose as follows: 1.013, 160 ml.; 1.014, 130 ml.; 1.015, 130 ml.; 1.012, 100 ml.; 1.009, 90 ml. Phenolsulfonphthalein excretion was 47 per cent in fifteen minutes, 90 per cent in two hours. Twenty-five serial urinalyses showed no albumin, and specific gravities ranged between 1.010 and 1.015 except for two specimens with recorded specific gravities of 1.020. No fat laden macrophages were seen in the urine sediment examined with polarized light. Two stools for occult blood were negative. Nose and throat culture

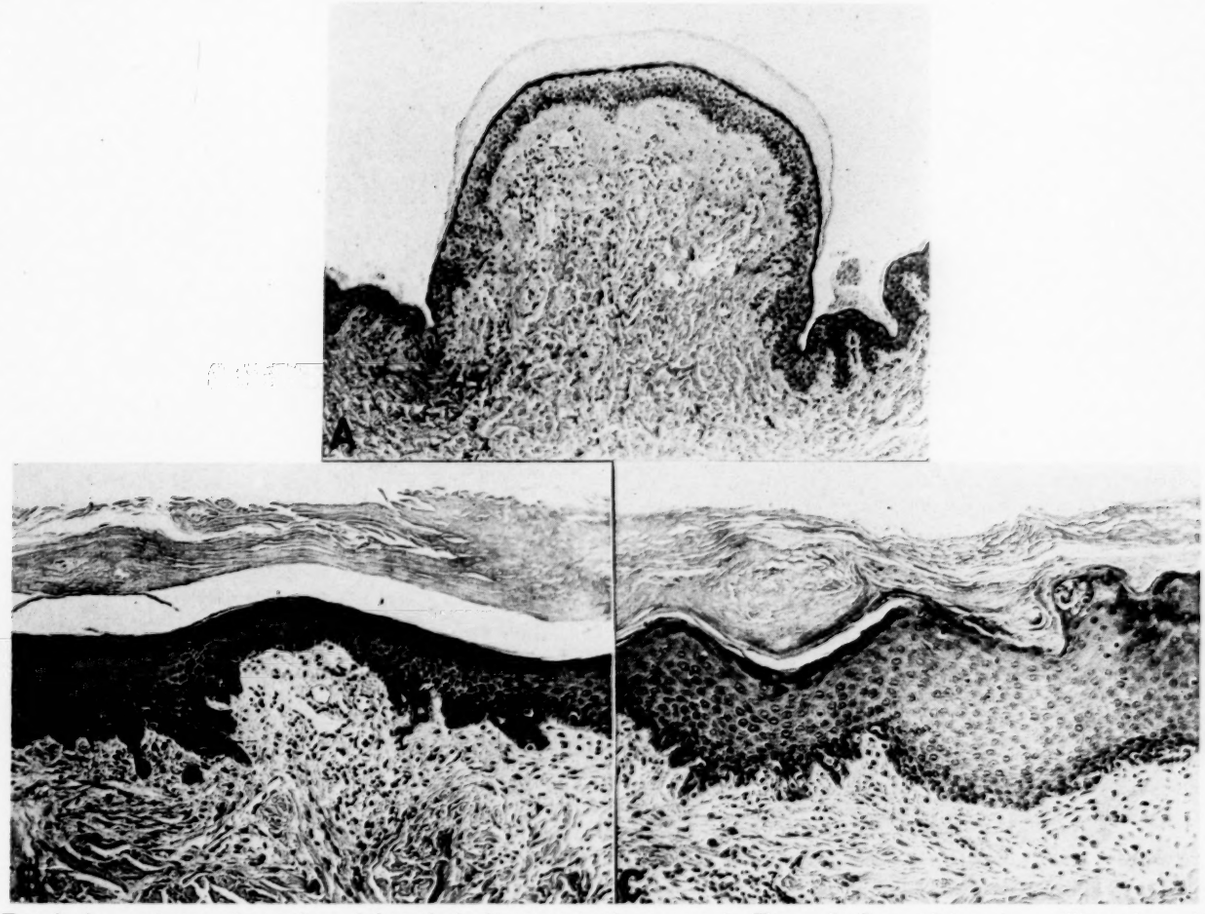


FIG. 3. A, a microscopic section of the relatively acute angioma seen in Figure 2. C, overlying the capillary dermal angioma is a rather clearly defined hyperkeratosis. The sclerosing older type of angioma with associated increased melanin deposition in the basal layers of the corium is demonstrated on microscopic section in B and C.

showed normal flora. Three separate urine cultures were negative. Four blood cultures taken during an episode of acute fever were negative. The roentgenogram of the chest showed questionable early changes of bronchiolitis with slight exaggeration of bronchovascular markings in the peripheral lung fields; however, this was regarded by some radiologists as a normal variation. Roentgenograms of the long bones revealed no abnormalities. The electrocardiogram initially showed an inverted T in leads II, III, aVF, and V_4 to V_6. Serial electrocardiograms showed gradual reversion of these T wave abnormalities to a normal pattern. The small deep Q wave seen in limb leads II and III (Fig. 1) remained unchanged.

Pulmonary function tests showed a vital capacity of 71 per cent and a maximum breathing capacity of 82 L./minute (62 per cent of normal). More detailed pulmonary function studies are shown in Table I. The lung volume studies performed indicated an increased residual volume. Ventilation values were within normal limits except for a rather low O_2 extraction per liter of air inhaled, i.e., 31 ml. O_2/L., the mean normal value being 46.8 plus or minus 7 ml./L. Distribution studies further substantiated this abnormality, with dead space ventilation 41 per cent of minute ventilation (normal value, 30 per cent). Diffusion studies revealed increased alveolar-arterial partial pressure differences of 37 mm. Hg on room air and 17 mm. Hg on 13 per cent O_2. Partial pressure of CO_2 was low normal, probably due to mild hyperventilation. Thus this patient's function studies revealed an increased residual volume which was associated with a distribution inefficiency resulting in a low O_2 extraction (mild emphysema). Associated with this there was an increased alveolar-arterial O_2 gradient (capillary-alveolar block). These abnormalities produced some desaturation of arterial blood. The persistence of the elevated alveolar-arterial gradient when breathing 13 per cent O_2 differentiated this syndrome from venous admixture (shunting) such as might result from multiple pulmonary infarcts.

During the entire hospital course the patient continued to have repeated small episodes of bright red hemoptysis. Dicumarol therapy was discontinued on the third hospital day. Because of the peculiar skin lesions (Fig. 3) it was believed that the most likely

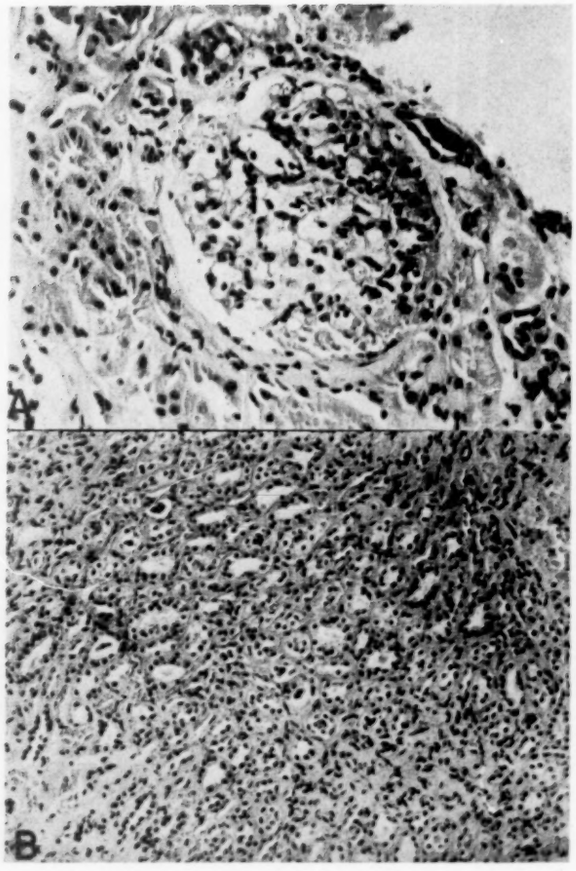


FIG. 4. Renal biopsy specimens shows an affected glomerulus with early thickening and hyalinization of Bowman's capsule and increased vacuolization of the tuft with accompanying thickening of the capsule in A. Distal tubules which are vacuolated with disruption of cellular integrity are shown in B.

diagnosis was angiokeratoma corporis diffusum universale (Fabry). Under our observation new areas of bright red angiomatous lesions developed which progressed over a period of seven to ten days to the more chronically appearing, flat, brown, pigmented lesions. The patient had intermittent substernal chest pain and required small doses of narcotics for relief. Skin biopsy specimens were obtained from both the bright red lesions and the more chronic appearing areas. These are shown in Figure 3. The sternal marrow examination was within normal limits, no fat laden macrophages being seen. The patient's polyuria, which measured between 3 to 5 L./day, was striking. Upon close questioning he stated that for several months he had noted decreased sweating over the upper region of the trunk. Subsequently, a sweat test was performed and demonstrated, by the starch iodide method, definite decreased sweating over the upper portion of the chest and regions of the arm, with normal sweating over the upper abdominal area. Sections from a renal biopsy specimen are shown in Figure 4. Unstained sections of the kidney biopsy specimen were found to have no fatty material on examination with polarized light or with Sudan Black staining, such as described by Pittekow et al. [*2*]. Biopsy of the left gastrocnemius muscle revealed no abnormalities. No vacuolated changes in the muscle or medial areas of small and medium-sized arteries were seen. In the area of the gastrocnemius biopsy intractable edema slowly developed, which was not relieved by elevation or elastic wrappings. The biopsy site also healed very slowly. This area of isolated peripheral edema remained for about two to three weeks and was associated with intermittent severe aching pains in that region. The patient also experienced severe, migratory, intermittent, aching musculoskeletal pains without obvious explanation. Two episodes of high fever occurred, with rectal temperatures up to 103°F., lasting for periods of three to five days without chills and without localizing signs. His white blood cell count remained normal during these episodes. Blood, throat and urine cultures were negative. Because of personal problems it became necessary to transfer him to a military hospital facility closer to his home.

COMMENTS

Angiokeratoma corporis diffusum universale (Fabry), A.C.D.U. was initially described in 1898 by Fabry as purpura hemorrhagica nodularis. It was later given the name of angiokeratoma by Mibelli. This disorder has been well summarized, with a report of the first American case and a review of the world literature including twenty-three other documented cases, by Fessas, Wintrobe and Cartwright [*1*]. Since then other reports have appeared, with further documentation of cases [*2–9*].

The principal manifestations are multiple skin angiomas, diffuse musculoskeletal pains frequently exacerbated by extremes of temperature, localized peripheral edema, dyspnea, emphysema, cardiomegaly with diffuse electrocardiographic damage, albuminuria, polyuria with low specific gravity, periodic fever, decreased sweating, a familial tendency, and a marked preponderance in males (only one case report of a female, diagnosed on the basis of autopsy material [*7*]).

The patient described herein is particularly interesting since he combines many of the reported features of the disease. To our knowledge no such multiplicity of system involvement has been observed in one patient. The lesions shown in Figure 3 are typical, with predisposition to the trunk regions. The raised angioma with fine overlying hyperkeratosis (Fig. 2C),

substantiated as a capillary angioma with hyperkeratosis (Fig. 3A), is characteristic. Rapid progression to the chronic, somewhat sclerosed lesion (Fig. 2D, 3B and 3C) is unusual and not previously reported. A fresh lesion appeared on the right foot (Fig. 2A) as a bright red raised angioma, with evolution to the sclerosed hyperpigmented form in several weeks.

In the kidney, the glomeruli (Fig. 4A) showed very early thickening of Bowman's capsule and early vacuolated changes in the capillary tuft. Vacuolization of distal tubular endothelium and disruption of cellular integrity were the only other pathologic renal changes seen. (Fig. 4B.)

These histologic findings have been previously well described, the vacuolated honey-comb appearance of glomeruli being most typical. The proximal tubules are characteristically spared, the most prominent involvement being of the loops of Henle and the distal tubules, in the form of vacuolation of cells. This patient did not show intimal thickening and medial degeneration with foam cells of small arteries and arterioles. Such lesions have been described [*4,7,8*].

This is the first case reported presenting a clinical picture of multiple pulmonary infarcts, although on close examination this diagnosis appears very unlikely. To have almost daily hemoptysis for four months with repeatedly normal findings on chest examinations, both physically and roentgenographically, would be most unusual. Pulmonary function tests were indicative of mild emphysema with alveolar-capillary block and did not show changes expected with pulmonary infarcts. More reasonably, the bleeding arose from angiomas in the bronchial tree (not previously described) or from damaged alveolar capillaries. These etiologies were never demonstrated and are only speculative. Hemoptysis has not previously been noted in A.C.D.U. The initial episode of chest pains and electrocardiographic changes probably represented acute myocardial damage, mostly to the posterior and lateral wall. Because of its acuteness this may have been secondary to a vascular occlusive episode, but underlying associated direct involvement of the muscle fibers by A.C.D.U. may have rendered them more susceptible to such an insult. The pattern of pain suggests an associated pericarditis. Electrocardiographic changes of myocardial damage are referred to in the literature but no electrocardiograms apparently have been published for examination.

Marked and incapacitating dyspnea, noted here, is described as a common feature in the literature [*1*]. This is the first documentation of a pathophysiologic alveolar-capillary block pattern, and certainly fits well with the symptom of progressive dyspnea. Eosinophilic alveolar deposits have previously been observed. Other characteristic observations, all previously described as part of this syndrome and manifested by this patient, include episodes of unexplained fever, localized peripheral edema, decreased sweating and diffuse musculoskeletal pains.

Preceding the cardiopulmonary manifestations this patient had evidence of distal tubular malfunction in the form of polyuria. This was later documented as marked inability to concentrate urine and conserve water even after combined water deprivation and pitressin administration.

The pathogenesis involves an abnormal lipid, probably a phospholipid, which has been noted in many tissues, including blood vessels, muscle, heart, ganglion cells, glomeruli and renal tubules. Vacuolated changes in these same tissues, with occasional deposition of hyaline amorphous material, have been found [*4*]. The medial musculature of small and medium-sized arteries and the capillary endothelium are chiefly affected. The lipid responsible for the vacuolization has been difficult to stain, requiring special technics [*4,5*]. The proliferation of capillary endothelium to form angiomas has not been seen outside of the dermis. This is unusual since capillary beds in other areas manifest the vacuolated changes. This may be a type of specialized reaction on the part of skin capillaries to an abnormal lipid, or the lipid abnormality and skin capillary proliferation may be controlled by the same genetic aberration. An irritative type phenomenon, however, is more particularly suggested in this case. The formation of scarring around the capillary proliferation, with accompanying melanin deposition (Fig. 3B and 3C), is a process which calls to mind the reaction of skin to varying forms of damage or foreign material (such as radiation, burns and abscess formation).

The differential diagnosis of the skin lesions includes angiokeratoma Mibelli, which usually occurs over bony protuberances; angiokeratoma scroti, which occurs on the scrotum; circumscribed angiokeratoma, which is localized; and purpura, which is usually peripheral. Angiokeratoma corporis diffusum universale shows

centripetal distribution, frequently with clustering of lesions around the umbilicus, buttock and scrotal region.

This is a rare familial disease of unknown etiology, with involvement of many organ systems and wide manifestations of symptoms and histopathologic findings. An abnormal lipid intimately related to small artery and capillary changes is of apparent importance in this disorder. The red spots on the skin, although not disabling, are the key to the diagnosis. Patients may live twenty to thirty years after the initial diagnosis and frequently symptoms do not develop until five to ten years after the initial skin lesions appear. They usually die of chronic renal failure, myocardial damage with acute myocardial infarction and congestive heart failure or of progressive respiratory failure. No specific treatment has been found.

SUMMARY

A case of angiokeratoma corporis diffusum universale, with particularly widespread system involvement and newly described features, is presented and discussed.

Acknowledgment: We wish to express appreciation to Dr. Elliot Rappaport and his staff at the Cardiopulmonary Function Laboratory, Mount Zion Hospital, San Francisco, California, for performing the detailed pulmonary function tests shown in Table 1; to Major Robert E. Pierce, MC, and his staff at the Histopathology Center, Travis Air Force Base, for help with tissue section preparations and special staining technics; and to Staff Sergeant K. M. Snyder and his staff at the Medical Illustration Department, Travis Air Force Base, for preparation of the photographic material.

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Effect of Induced Pneumothorax on the Pulmonary Shunt and the Ventilation in a Patient with Atelectasis of the Lung*

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The effect of the state of lung inflation, and especially of atelectasis of the lung, on pulmonary vascular resistance has been a matter of debate. Recently Whittenberger et al. [*1*] demonstrated that acute atelectasis in open chest dogs produces little or no increase in pulmonary vascular resistance; hyperinflation on the other hand is accompanied by a marked increase in pulmonary vascular resistance. The present data from a patient agree with these results. They also demonstrate that the shunt through an atelectatic lung may be much higher during exercise than at rest. Pneumothorax was induced on the side of the atelectasis in an attempt to reduce the shunt.

CASE REPORT

The patient, a forty-one year old woman, was treated in 1955 for tuberculosis of the left lung. She had a "cold" in February 1959, eleven months before the admission; her main symptom was a non-productive cough. She had another bout of this "cold" four months later and was admitted to the local sanatorium. There were parenchymal lesions in the upper lobe of the left lung, and a small focus in the upper lobe of the right lung. Bronchoscopy two months later showed a tuberculous bronchitis in the bronchus of the upper lobe of the left lung. She was treated with streptomycin, para-aminosalicylic acid and isoniazid, later also viomycin. There was a good response.

On two occasions, two months prior to admission to this hospital and five weeks later, she had had a feeling of compression and pain in the left side of the chest when going to bed. On both occasions chest roentgenograms showed a complete atelectasis of the whole left lung. On the first occasion the findings on chest roentgenogram gradually became normal and the symptoms disappeared after a few days. Bronchoscopy revealed complete obstruction of the left main bronchus at the level of the orifice of the upper lobe. She was sent to this hospital for evaluation for operation.

On admission, on January 12, 1960, there was no dyspnea or cyanosis at rest. The blood pressure was 120/80 mm. Hg and the heart rate was 130, with sinus rhythm. Over the whole left side of the chest there was marked dullness. The breath sounds and the movements of the chest wall were much weaker on the left side than on the right. At the base of the left lung there were no breath sounds, and at the apex there were bronchial breath sounds. A chest roentgenogram revealed that the left lung was totally collapsed, with marked displacement of the trachea and the mediastinum to the left. Part of the right lung was seen in the left half of the chest. (Fig. 1A.)

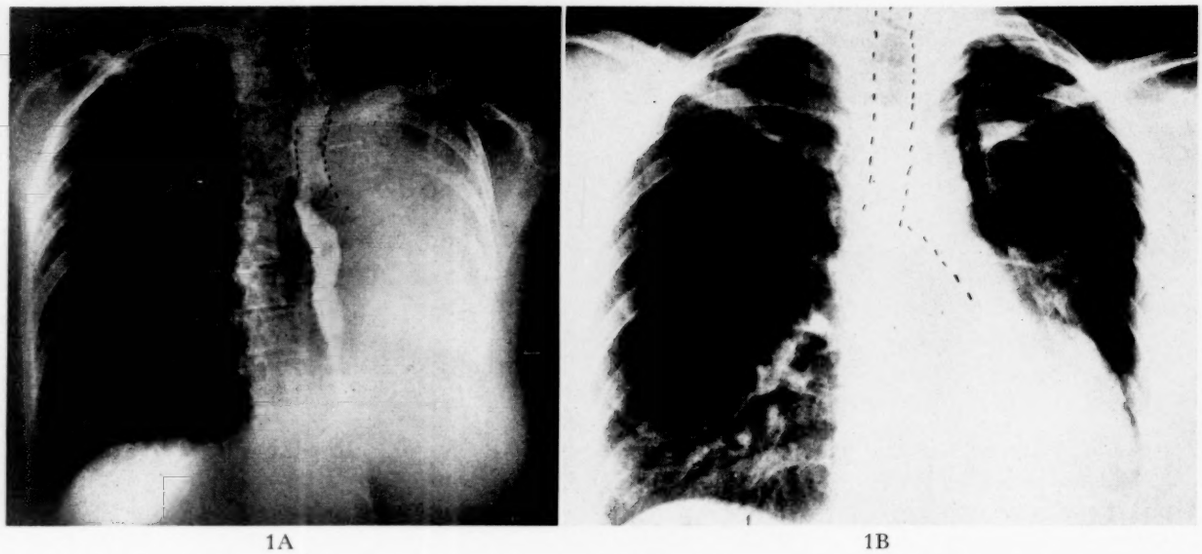
Bronchoscopy revealed, as before, that the left main bronchus was almost totally occluded at the level of the orifice of the upper lobe. There was an opening the size of a pin point, in which there was an air bubble, which hardly moved on breathing. It seemed to be a stenosis due to scarring but with no ulceration.

It was noted that the patient became very dyspneic and cyanotic when walking slowly. The patient was therefore examined in the pulmonary function laboratory. As will be shown, the data indicated that the patient had a venoarterial shunt. On exercise this shunt apparently increased and there was marked cyanosis and hyperventilation.

In an attempt to decrease the shunt through the atelectatic lung a pneumothorax was induced on the side of that lung, and the studies were repeated the following day. The data indicated a marked improvement. The patient felt much better and had no further symptoms of dyspnea.

The patient was operated upon after having had pneumothorax for ten days. The left lung was found to be totally collapsed and there were small adhesions at the apex and at the base. Tuberculous lesions were found only in the upper lobe. The upper lobe, with the lingula, was resected. No lesions could be palpated

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1A 1B

FIG. 1. Chest roentgenograms. A, before institution of pneumothorax. B, after institution of pneumothorax.

in the lower lobe. Three centimeters of the main bronchus were resected and a wide end-to-end anastomosis was made between the bronchus of the lower lobe and the left main bronchus. After the operation there was a small exudate on the left side of the chest which gradually disappeared. Three weeks after the operation the patient was sent back to her local sanatorium.

METHODS

The ventilatory capacity was measured with a Bernstein [*2*] spirometer, and was compared with normal values obtained with the same equipment [*3*]. The terminology recommended by Gandevia and Hugh-Jones is used [*4*].

Arterial blood samples were obtained from an indwelling polyethylene catheter as described by Seldinger [*5*]; this catheter was left in the left brachial artery for three days. The O_2 saturation was determined spectrophotometrically using the technic developed by Ställberg-Stenhagen and recently published by Holmgren and Pernow [*6*]. The pH of the blood was determined with a temperature-controlled glass electrode pH-meter (Radiometer, Copenhagen). The CO_2 tension was obtained by use of the pH value and the pH values obtained after equilibrating two aliquots of the blood with gases of known CO_2 tensions [*7*].

The minute ventilation at rest and exercise was measured by collecting expired air in a Tissot spirometer with a recording kymograph. The expired gas was analyzed for O_2 and CO_2 in a Scholander [*8*] micro gas analysis apparatus. Physiologic dead space was calculated from CO_2 tensions in arterial blood and expired air, using the Bohr formula [*9*]. The terminology for respiratory measurements recommended by Pappenheimer et al. [*10*] is used.

TABLE I
VENTILATORY TEST

Test	Observed	Normal	
		Mean	Mean −2 S. D.
Vital capacity	3.1	3.8	2.9
Forced vital capacity	2.9		
Forced expired volume in 1 second	2.3	2.9	2.2
Forced expired volume %	81%	85%	72%
Maximal voluntary ventilation (rate 40 breaths/min.)	84	100	72

NOTE: All volumes are expressed in liters at body temperature, ambient pressure saturated with water.

RESULTS

Studies During Atelectasis, without Pneumothorax. The ventilatory capacity data are shown in Table I. They were all lower than the mean of the values obtained in normal subjects of the same age group, but were within normal limits [*3*]. The alveolar N_2 gradient, obtained by a modification of Fowler's single breath test, was normal [*11*].

Blood gas and ventilation studies were performed with the patient supine, in the left and right lateral positions, and sitting on a bicycle ergometer at rest and during mild exercise (200 kg./minute). The data are shown in Table II.

At rest the arterial O_2 saturation was slightly below normal, 92.5 per cent sitting and 94.4 per cent supine (not shown in table). The saturation in the left lateral position was lower than in

TABLE II
STUDIES BEFORE AND DURING PNEUMOTHORAX IN A PATIENT WITH ATELECTASIS

Study	Atelectasis of Left Lung					Atelectasis of Left Lung plus Pneumothorax			
	Lateral Positions	Rest Air	Exercise Air	Rest 50% O_2	Exercise 50% O_2	Lateral Positions	Rest Air	Exercise Air	Exercise 50% O_2
$\dot{V}_{O_2}$ ml. STPD	...	253	750	...	...	...	254	673	...
Sa_{O_2} %	R 97.7 L 92.3	92.5	74.0	99.7	81.7	R 95.7 L 94.9	94.5	95.2	99.8
Pa_{CO_2} mm. Hg	...	35	25	...	30	...	39	36	39
pH	...	7.50	7.53	...	7.49	...	7.45	7.45	7.43
$\dot{V}_E$ 1 BTPS	...	8.0	38.5	9.5	...	R 4.0 L 6.9	8.3	18.8	16.8
Breaths/minute	...	19	37	20	28	R 8 L 16	21	20	20
V_T 1 BTPS	...	0.41	1.03	0.46	...	R 0.48 L 0.42	0.39	0.93	0.83
V_D 1 BTPS	...	0.15	0.37	...	...	...	0.16	0.22	...
Heart rate	...	101	122	...	128	...	101	110	110

NOTE: All the data except those in the "lateral positions" were obtained with the patient sitting on the bicycle ergometer. $\dot{V}_{O_2}$ = O_2 uptake per minute. Sa_{O_2} = arterial O_2 saturation. Pa_{CO_2} = arterial CO_2 pressure. $\dot{V}_E$ = minute ventilation. V_T = tidal volume. V_D = physiologic dead space. R = right; L = left.

the right lateral position. The saturations after five, ten and fifteen minutes in the lateral position were left 93.4, 92.3, and 92.3 per cent, and right 94.4, 96.9, and 97.7 per cent, respectively.

During exercise the O_2 saturation fell markedly and the patient was cyanotic as well as dyspneic. When breathing 50 per cent O_2 she was well saturated at rest but was still markedly unsaturated during exercise. The O_2 capacity of the blood was 16 volumes per cent. The calculated shunt at rest and exercise were 10 to 15 per cent and 30 to 35 per cent of the cardiac output, respectively (assuming a-v O_2 differences of 3.5 and 6.5 volumes per cent).

There was hyperventilation, moderate at rest and very marked on exercise. Also the alveolar ventilation was very high as indicated by the low CO_2 tension. The lowest CO_2 tensions were observed when the arterial blood was most unsaturated. The dead space increased during exercise as did the respiratory rate.

Studies During the Insufflation. Eight hundred milliliters of air were insufflated through a needle in the sixth left interspace, with the patient supine. Meanwhile the ventilation was recorded on a bag-in-box spirometer. This recording showed a lung volume decrease of 350 ml.; thus 450 ml. of the insufflated air were used for chest expansion.

A chest roentgenogram after the pneumothorax showed that the collapsed lung had an adhesion at the apex; that there was air medial to the left lung; and that there was now some air in the upper lobe. There was also a little fluid in the thorax. (Fig. 1B.)

Studies after Induced Pneumothorax. These are also shown in Table II. Figure 2 shows a comparison of the values before and during pneumothorax. The arterial O_2 saturation at rest and exercise were within normal limits. The high arterial saturation during exercise, with air and with 50 per cent O_2, indicates that there was practically no shunt, not even during exercise. The calculated shunt during exercise was less than 5 per cent of the cardiac output (assuming an a-v O_2 difference of 6 volumes per cent). There was much less hyperventilation than before, as is demonstrated by the figures for minute ventilation during rest and exercise and by the arterial CO_2 tensions, which were nearly normal.

There are some additional comparisons of findings before and after the pneumothorax: (1) After the pneumothorax the arterial O_2 saturations in the lateral positions differed much less than before the pneumothorax. (2) The minute ventilation during identical exercise was twice as large before the pneumothorax as

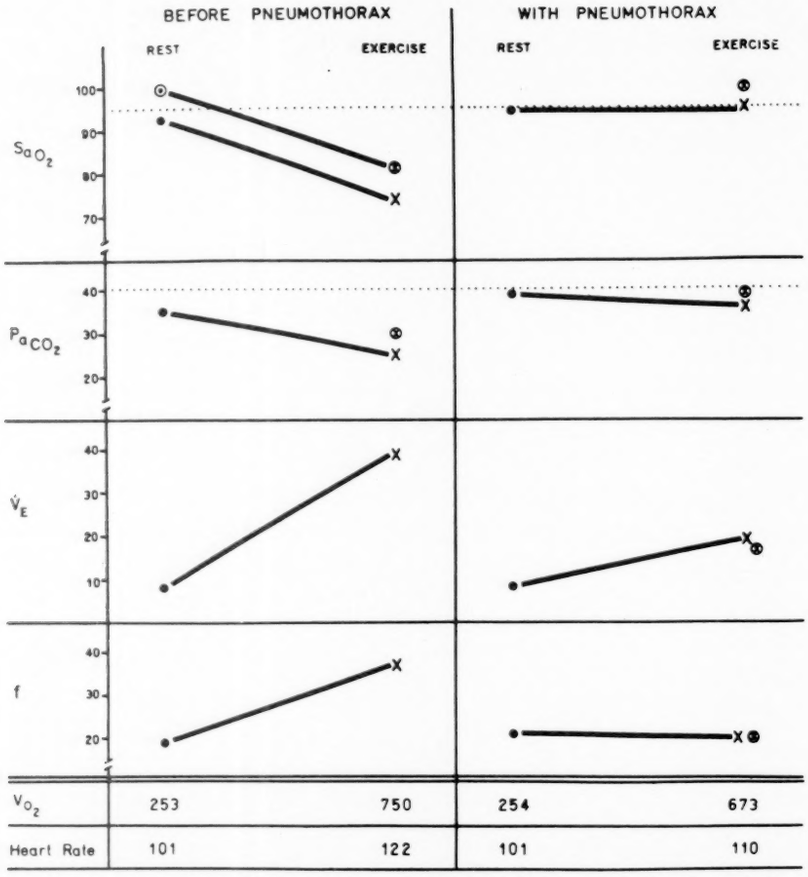


FIG. 2. Comparison of blood gas and ventilation data before and after institution of pneumothorax. All the data were obtained with the patient sitting. For key to symbols see Table II. ●● denotes resting values, breathing room air, ⊙⊙ denotes resting values, breathing 50 per cent O_2, ×× denotes exercise values, breathing room air, ⊗⊗ denotes exercise values, breathing 50 per cent O_2.

after it. Similarly the respiratory rate during exercise (with or without extra oxygen) was much higher before the pneumothorax than after. (3) At rest the oxygen uptake per minute was the same before and after the pneumothorax. With identical exercise the oxygen uptake was markedly higher before pneumothorax than after. (4) The heart rate at the same level of exercise was higher before the pneumothorax than after.

COMMENTS

This patient presented the picture of total or subtotal atelectasis of the left lung, due to a bronchial occlusion. On slight exercise she became markedly dyspneic and cyanotic. Her ventilatory capacity was within normal limits, and her symptoms during exercise were apparently due to shunting through the atelectatic lung and to increased work of breathing.

Lung Volumes and Transpulmonary Pressures during Unilateral Atelectasis. In the totally atelectatic lung the transpulmonary pressure, i.e., the difference between the pressures in the alveoli and outside of the lung, approaches zero. The extrapulmonary (intrapleural) pressure is more negative than normal and the intrapulmonary pressure also is below that of the atmosphere.

The decreased volume of the atelectatic lung is compensated for by increased volume of the contralateral lung and by decreased volume of the thorax. Dale and Rahn [*12*] found, in an elegant experimental study, that these two factors contribute approximately equally to volume compensation. Analogous conditions were found in artificial pneumothorax by Björkman [*13*] and Birath [*14*]. This relation may, however, be different in patients with variations in the compliance of the open lung and/or the chest wall, including the diaphragm.

In the present case it was evident that the right lung was markedly hyperinflated during the period of atelectasis of the left lung, so much as to present the picture of "right lung hernia" into the left hemithorax. There was no indication of decreased compliance of the thoracic cage, and one may conclude that the right lung possessed good distensibility.

Resistance to Blood Flow in the Atelectatic and the Hyperinflated Lung. The fraction of the cardiac output going through the atelectatic lung depends on the relative resistances in the two lungs; it is thus important to consider the resistances in the two lungs separately, and to consider how these will change during exercise.

Whittenberger et al. [*1*] have recently published an experimental study on the pulmonary vascular resistance at various levels of lung inflation, ranging from complete atelectasis or collapse to full inflation. " . . . the vascular resistance in the lung at collapse is usually only slightly higher than the resistance at an intermediate level of filling." A moderate increase of the vascular resistance at collapse sometimes occurred at lower levels of blood flow—levels such as might be present in a patient at rest. This is illustrated in the *upper* curve of Figure 3. The "collapse" resistance is the highest point at the left; the lowest part of that curve would correspond to a normal functional residual capacity and transpulmonary pressure. The vascular resistance in the contralateral lung must be on the right ascending part of the upper curve, because that lung is hyperinflated; it may therefore be as high as in the collapsed lung or even higher.

In the closed chest the perialveolar vessels in the functioning lung are exposed to a pressure which is near that of the external atmosphere at the end of inspiration and expiration. In the atelectatic lung, on the other hand, they are exposed to a pressure which is always more negative than in the open lung. The perialveolar vessels in the atelectatic lung are therefore more distended and have a lower flow resistance. In this respect the atelectatic lung differs from the lung during pneumothorax [*15*]; this is one of the reasons why the atelectatic lung shunts more blood than the lung subjected to pneumothorax.

During exercise the cardiac output and the pulmonary artery pressure are higher than at rest. This causes increased distention of the pulmonary vascular bed and the conditions determining vascular resistance become more

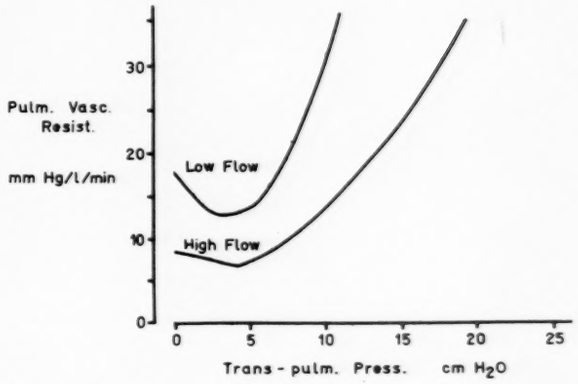


FIG. 3. Relation between transpulmonary pressure and pulmonary vascular resistance.

similar to those depicted in the *lower* curve of Figure 3 [*1*]. Furthermore, there is probably still more hyperinflation of the functioning lung than at rest. Each of these two factors, the increased vascular distention and the increased inflation of the functioning lung, will increase the proportion of the cardiac output going through the atelectatic lung. This, together with the lower O_2 content of the shunted venous blood, causes increased hypoxemia during exercise.

With time, the shunt through an atelectatic lung usually diminishes and may practically disappear within a few weeks [*16–18*]. This must be due to active vasoconstriction in that lung, probably because of local hypoxic stimulation [*19*]. It was surprising to find such a large shunt in this patient; we cannot, however, be sure that the atelectasis had been complete and uninterrupted during the five weeks between its detection and the study.

Effect of Pneumothorax on the Blood Flow Through the Two Lungs. Although no detailed reports are available on the conditions of the pulmonary circulation during pneumothorax, unilateral or bilateral, it is likely that the blood flow through the collapsed lung during pneumothorax is reduced. For this and the following reasons it was presumed that pneumothorax might diminish the blood flow through the atelectatic lung, during exercise as well as at rest: (1) the hyperinflation of the contralateral lung would be reduced, and this would diminish the resistance in that lung [*1*]; (2) the pressure to which the perialveolar vessels in the atelectatic lung are exposed would become less negative, and this would raise the resistance in these vessels.

The findings after induction of the pneumothorax seem to confirm these assumptions. While

the shunt during exercise was 25 to 30 per cent of the cardiac output before pneumothorax, it was less than 5 per cent after pneumothorax was instituted.

A similar case of atelectasis of the lung with venoarterial shunting and improvement with artificial pneumothorax has previously been published by one of the authors [*20*]. Between the fillings of the pneumothorax the patient complained of increasing dyspnea and showed increasing cyanosis. A study immediately before insufflation revealed an arterial O_2 saturation of 78 per cent. A few minutes after the insufflation the O_2 saturation was 87 per cent. The ventilation was 9.3 L./minute before, and 7.7 L./minute after the insufflation. Respiratory rates were thirty-four and twenty-five per minute, respectively. The effect of pneumothorax was interpreted in the following way: "When insufflation of air is carried out and the strong negative pressure in the pleural cavity is relieved, these opened vessels (within the atelectatic parts) collapse once more, and the oxygen-saturation rises."

A similar comparison of the gas exchange before and during induced unilateral pneumothorax in a patient without atelectasis was recently made in this laboratory. There was no change in the O_2 saturation. The only difference observed was a lower tidal volume and a higher breathing rate at higher exercise levels during the period of pneumothorax.

Effect of Body Position on the Blood Flow Through the Atelectatic Lung. There was a definite difference between the arterial saturations in the left and right lateral positions; apparently more blood passes through unventilated areas in the left lung when that lung is dependent. This is in agreement with the findings of Mattson and Carlens [*21*], and others [*22*], and is due to the fact that vascular resistance decreases with increased hydrostatic pressure.

Minute Ventilation, Respiratory Rate and Work of Breathing. Before the pneumothorax, there was alveolar hyperventilation, as reflected by the low arterial CO_2 tensions. This was especially marked during exercise. The tidal volumes at exercise were of the same magnitude before and after pneumothorax. The very large minute ventilation on exercise before the pneumothorax was achieved mainly by means of a high respiratory frequency.

That the hyperventilation must be due to hypoxic drive is indicated by the values on exercise when the patient was breathing room air or 50 per cent O_2, before and with pneumothorax. The values for minute ventilation and respiratory frequency increased with decreasing arterial O_2 saturation, despite the fall in arterial CO_2 tension and the rise in arterial pH. This is in partial agreement with the findings of Dale and Rahn [*12*] in experimental atelectasis. With the vagi intact, the increase in ventilation in their experiments was not, however, large enough to prevent acidosis. This could possibly be explained by the effect of the anesthesia. Barr et al. [*23*] observed marked hyperventilation with respiratory acidosis during pulmonary shunting in dogs exposed to radial acceleration. The increased chemoreflex drive in those experiments resulted from reduction in blood flow to chemoreceptors as well as from the generalized hypoxemia. The role of stretch receptors in the lungs and thorax is difficult to evaluate.

The physiologic dead space at rest was the same before and during pneumothorax, when the respiratory rates were equal. The high values during exercise, especially high before pneumothorax, seem to relate to the respiratory rate.

The work of respiration during exercise before institution of the pneumothorax, with a minute ventilation and breathing rate approximately twice as high as after pneumothorax, was probably at least three times as great as during the same exercise after induction of the pneumothorax [*24*]. Another factor which tends to increase the work of breathing is the use of only one lung. The patient's effective lung compliance must be lower than normal [*25*].

Oxygen Consumption and Heart Rate. During exercise on the bicycle ergometer the O_2 consumption was higher before the pneumothorax than during the same exercise with pneumothorax. The difference is probably attributable, at least to a great extent, to the increased work of breathing. This is in agreement with data obtained by Dale and Rahn [*12*] during experimental atelectasis of the lung.

In addition, the heart rate at exercise was more rapid before pneumothorax than after. If this difference is significant, it is most likely related to the difference in O_2 consumption. The effect of hypoxemia on the heart rate must, however, also be considered.

SUMMARY

A patient with atelectasis of the left lung due to tuberculous stenosis of the left main bronchus

was studied at rest and during mild exercise. There was moderate arterial hypoxemia at rest and marked hypoxemia upon exercise. The hypoxemia was accompanied by alveolar hyperventilation. The arterial O_2 saturation was markedly lower when the patient lay on the left side than on the right side; this difference was fully evident after ten minutes in a lateral position.

On the basis of previous data in animal experiments it was assumed that induction of pneumothorax on the side of the atelectasis might decrease the shunt through the atelectatic lung, during exercise as well as at rest. Eight hundred milliliters of air were insufflated in the left side of the chest.

With the induced pneumothorax the patient had normal arterial O_2 saturation during exercise as well as at rest. The shunt through the left lung at exercise was reduced from 30 to 35 per cent to less than 5 per cent of the cardiac output. The ventilation and respiratory rate at exercise were approximately half as great as before the pneumothorax. The alveolar ventilation was within normal limits.

The patient was later operated upon; a lobectomy and a bronchoplasty were performed with no complications.

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Acromegalic Gigantism*

The Buffalo Giant

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ACROMEGALY is a not infrequent endocrine disorder, occurring in approximately 1:3,000 hospital admissions [*1*]. Pituitary gigantism is much less frequently seen, although its exact incidence is not clearly established because of the relatively few cases reported in the literature. It is a well known fact, however, that in approximately 40 to 50 per cent of cases of gigantism due to pituitary dysfunction, acromegalic features also are present [*2,3*].

Recently, we had the opportunity of studying an acromegalic giant. A review of the literature showed that Dana [*4*], in 1893, published a report on the giant named Wilkins. Bassoe [*3*] reported the same case in 1903, with postmortem findings. Even earlier, in 1878, Buhl [*5*] recorded his observations on a giant named Thomas Hasler. Museum skeletons of giants in Dublin, St. Petersburg and London were known even before then [*3*]. Hutchinson [*6*] was probably one of the first to report a case of acromegalic gigantism in a female patient, and in 1898 proposed his theory as to the probable role of the pituitary gland in acromegaly and gigantism [*7*].

The twentieth century saw more case reports recorded in the literature [*8–18*]. In the past decade, however, little seems to have been added to our knowledge of this disease. Recently, however, Gershberg et al. [*1*] have commented on some aspects of renal function in acromegalic gigantism. Wermer [*19*] and Underdahl et al. [*20*] have noted the association of multiple endocrine adenomas with pituitary tumors.

Hurxthal [*21*] and Soffer [*2*] in their textbooks and McFarland [*22*] in his treatise on gigantism give excellent descriptions of the clinical and pathologic findings noted in some of the famous giants of the past. However, laboratory data in many areas of study were not clearly defined and in some aspects were completely lacking.

The present case report has many interesting features, the first being that the patient had not received previous treatment up to the time of this report; secondly, there is evidence of continued growth activity. An attempt was made to study the various endocrine glands for evidence of their degree of activity, yielding data not previously reported. The fact that this disease was allowed to progress without specific therapy brings out the natural history of the disease, with its numerous complications.

CASE REPORT

The patient (J. C.) first came to our attention on October 14, 1959, after having fallen at home sustaining an injury to his right foot and ankle.

A review of this patient's history showed that he was a twenty-seven year old white man of Irish descent who weighed 9 pounds 5 ounces at birth. The pregnancy and delivery were uncomplicated. From his history and from studying pictures, the patient apparently enjoyed good health during infancy and childhood. From early childhood it was noted, however, that he was always a little taller than his playmates of the same age, and that at the age of twelve years he had attained a height of 5 feet 6 inches, being the tallest member of his grammar school graduating class. (Fig. 1.) At sixteen years of age he was 6 feet 2 inches tall, very muscular, well coordinated in movement, popular with his classmates and well appreciated on his high school basketball team. His only complaints at that time were severe pains in the calves of his legs which would incapacitate him at times and force him to go to bed. He continued to enjoy good health, however, became noted for his tremendous strength (he could lift a car with both hands), and between the ages of sixteen and twenty years the pain in his legs gradually subsided.

In the space of a few months he had a very active growth spurt so that at approximately seventeen years

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FIG. 1. Patient at age twelve, 5 feet 6 inches in height.



FIG. 2. Patient at twenty-two years of age, height 7 feet 6 inches. The gentlemen flanking the patient are approximately 5 feet 11 inches in height.

of age he had attained a height of 6 feet 9 inches. His growth continued, and at the age of twenty he had attained a height of 7 feet. He denied ever having any body hair, has never had to shave more than once or twice a month and then only to remove soft, fuzzy growths of beard. Both axillary and pubic hair have always been scanty. He denied any sexual drive, libido or ever having had an erection or nocturnal emissions. There was no history of headache or visual disturbances. In his late teens he noted that his glove size had increased from approximately size 8 to one which was not available on the commercial market. His shoes had had to be custom made since he was sixteen years old.

Between the ages of twenty to twenty-two years he noted for the first time a gradual loss of muscle tone and strength, especially in his legs. A kyphoscoliotic deformity of his spine had begun to manifest itself. Despite the onset of kyphoscoliosis, his height at twenty-two years of age was reported as 7 feet 6 inches, an increase of 6 inches in a space of two years. (Fig. 2 and Table I.)

He is extremely well liked by all who meet him and he has made an excellent adjustment to life. At one time he ran for alderman in his political district, losing by only a few votes, while running his entire campaign from a hospital bed. He has driven a car since his late teens and even now, despite his orthopedic problems, drives a car with ease. However, because of his height he cannot drive from the usual position in the front seat; he has removed the front seat of the car and drives from the rear. He has a keen sense of humor, is of high normal intelligence and well versed in topics of current general interest.

His parents and one sister are of normal height and stature and in good health. A brother died at the age of five years with a perforated appendix and peritonitis.

On the maternal side of the family, a great grandfather was reportedly 7 feet tall. His maternal grandfather was 6 feet 6 inches in height and died of unknown causes at the age of seventy-three. The

TABLE I
SUMMARY OF GROWTH OF PATIENT (J. C.) SINCE THE AGE OF TWELVE

Age (yr.)	Height	
	Inches	Centimeters
12	66	167.6
16	74	188.0
20	84	213.4
22	90	228.6
24	90	228.6
27	96	243.8



FIG. 3. Roentgenogram of left knee taken when the patient was twenty-four years old demonstrating marked femoral subluxation and unfused epiphyseal plate of distal femur.

grandmother was 6 feet 2 inches tall and died at the age of sixty-eight of unknown causes. Six maternal uncles were all between 6 feet 2 inches and 6 feet 6 inches in height. Six maternal aunts were between 5 feet 8 inches and 6 feet in height.

The fraternal great grandfather was reportedly 7 feet 6 inches in height; he died in later life of unknown cause. A fraternal great uncle was 7 feet 2 inches tall. The grandfather was 6 feet 4 inches tall and died of unknown cause at the age of forty. The grandmother was of normal height and stature. Ten fraternal aunts and uncles are of normal height and stature.

The patient was first admitted to this hospital in July 1954 for removal of infected teeth. He was then twenty-two years of age, weighed 375 pounds and was 7 feet 6 inches tall. No symptoms were noted on the chart. Physical examination at that time disclosed the features of acromegalic gigantism, a small penis and epispadias. Except for a trace of albumin, the urinalysis and hemogram were within normal limits.

The patient was readmitted three months later for the second time with a comminuted fracture of the right femur sustained when his car rolled over on him while he was in the process of trying to repair it. His height and weight had not changed. His strength was alluded to at this time, in that he related how he held the car up with his hands, freeing his leg from beneath the rear wheels before help arrived. The examining physician noted at this time a diffusely enlarged nontender thyroid and bilateral gynecomastia. The radiologist noted that there was incomplete fusion of the distal femoral epiphysis bilaterally. Urinalysis revealed 4 to 6 white blood cells per high power field and 1 to 3 red blood cells per high power field but no albumin. The hemogram was again within normal limits. Treatment consisted of the insertion of two intramedullary nails into the right femur and the patient was discharged improved after two months' hospitalization.

The patient was then not seen until October 1956 when he was admitted at the age of twenty-four years with a pneumonic process in the lower lobe of the left lung. His height was not recorded at that time. Cardiomegaly was noted, with the apex in the seventh intercostal space at the anterior axillary line. There were no murmurs or gallop. An electrocardiogram disclosed an incomplete right bundle branch block and inverted T waves in leads III, aVF, V_1, V_2 and V_3. Roentgenograms of both knees again showed incomplete fusion of the distal femoral epiphyseal lines and marked anterior subluxation of the femurs. (Fig. 3.) The radiologist also noted a poorly defined trabecular pattern and relative demineralization, the cortical borders of the long bones appearing very thin.

He was treated with antibiotics, expectorants and analgesics, with marked improvement and was discharged on the sixth hospital day.

The patient was readmitted five months later (February 1957) with a history of having fallen on the ice. Roentgenograms revealed an intertrochanteric fracture of the left femur and a linear fracture of the ulna. The findings on physical examination had not changed except that gynecomastia was not noted on this admission. The hip was nailed and an attempt to correct the femoral subluxation on the left was made with a corrective osteotomy and patellectomy. Finally the patient was fitted with a long legged brace to his left leg.

His hospital stay at this time was stormy with development of thrombophlebitis in the calf of the left leg, pulmonary embolus, congestive failure and recurrent episodes of pyelonephritis. A large staghorn calculus in the right kidney was noted at this time. The subluxation of both femurs became more pronounced, and a bilateral peroneal palsy with foot drop then became evident. He was finally discharged thirteen months after admission.

The patient was next seen in October 1959 for the fifth time (present admission) with a history of having twisted his right ankle. Roentgenograms disclosed a linear fracture of the right talus. He had no complaints except those referable to the right ankle and generalized weakness for the past four years.

Physical examination disclosed a very tall, angular, skeletally deformed white man, twenty-seven years of age (Fig. 4), with typical features of acromegaly on a giant frame. (Table II.) The blood pressure was

TABLE II
PHYSICAL MEASUREMENTS OF PATIENT (J. C.) AT TWENTY-SEVEN YEARS OF AGE

Weight	325	
Height	96	(243.8)
Crown to pubis	45	(114.3)
Pubis to heel	51	(129.5)
Span	102	(259.0)
Head circumference (occipital to frontal)	26	(66.0)
Chest at mammary line	45	(114.3)
With expansion	48	(121.9)
Circumference at umbilicus	40½	(102.9)
Circumference at iliac crest	42½	(107.9)
Circumference at trochanters	43	(108.2)
Length of clavicle	9	(22.9)
Acromion to tip of third finger	43½	(109.4)
Length of hand from base of third metacarpal to tip of third finger	10¼	(26.0)
Length of third finger	6	(15.2)
Length of feet	11¾	(29.8)
Shoe size	16 EEEE	

NOTE: With the exception of weight, which is given in pounds, all other measurements are given in inches followed by the measurement in centimeters (in parentheses).

110 mm. Hg systolic and 70 mm. Hg diastolic. The radial pulse was 76 beats per minute and regular. Respirations and temperature were normal. The skin was smooth, soft and non-elastic, with prominent subcutaneous fat which had a doughy consistency. There was no evidence of hypertrichosis except on the dorsum of the feet and between the toes. There was normal distribution of scalp hair but only fine, light hairs on the skin of the face and chin. Axillary hair was scanty and the pubic hair had a female pattern. There was no hair on the chest or abdomen. The face had prominent bushy eyebrows with well delineated supraorbital ridges, a large wide nose and mandibular prognathism. The eyes, visual fields and results of funduscopy were entirely within normal limits. The lips were thick and large. The palate was high and arched. The tongue was thick, large and coated.

The thoracic spine showed a marked kyphoscoliosis, there was a pectus excavatum with flaring and protuberance of the costochondral junctions bilaterally and a narrowed xiphoid-costal angle (17 degrees). The lungs were clear to percussion and auscultation and the diaphragmatic excursion was within normal limits. The cardiac apex was in the seventh intercostal space at the anterior axillary line and there were no palpable heaves, kicks or thrusts and no precordial bulge. The first apical sound was louder than the second; the second pulmonic sound was equal to the second aortic sound and no split sounds were heard. A grade 2, early ejection type systolic murmur was heard along the left sternal border, most prominently at the third left intercostal space. There were no diastolic murmurs or gallops.

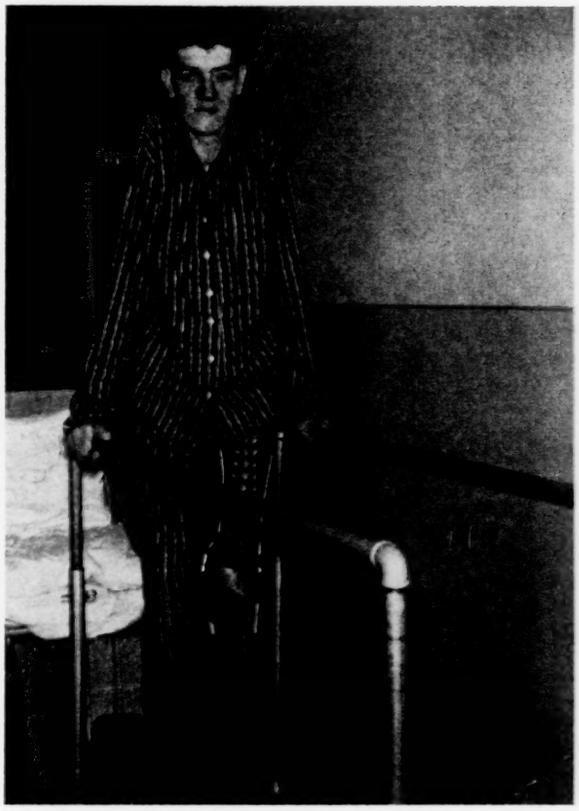


FIG. 4. Patient at twenty-seven years of age, 8 feet in height.

The abdomen was soft and scaphoid. The liver was palpable 2 cm. below the right costal margin in the mid-clavicular plane. The spleen and kidneys were not palpable. Bowel sounds were active. There was no costovertebral tenderness.

The arms were long, bony and awkward in appearance. There was a moderate degree of muscle atrophy, especially of the extensor muscles and shoulder girdles. The hands were gigantic, long and wide with slight fusiform appearance at the middle interphalangeal joints. There was marked hyperextensibility at the metacarpal-phalangeal joints and extensive atrophy of the interosseous muscles. The legs showed marked atrophy of the muscles in both calves and thighs, bilateral anterior subluxation of the femurs at the knees, an absent left patella and bilateral foot drop with huge club-like feet.

Rectal examination revealed a normal sphincter tone, but the prostate gland was not palpable. The scrotum appeared normal. Both testicles were small and firm. There was normal consistency of the epididymis and vas deferens bilaterally.

Roentgenograms of both hands in the anteroposterior projection (Fig. 5) revealed evidence of marked increase in length of the metacarpals and phalanges, with the typical increase in size of the distal tufts of the distal phalanges, characteristic of acromegaly.



FIG. 5. Hands. Note gigantic hands and tufting of terminal phalanges.

There also appeared to be an increased amount of soft tissue surrounding the proximal and middle phalangeal joints. Bony mineralization appeared to be satisfactory.

Roentgenographic examination of the feet (Fig. 6) revealed marked subluxations of the metatarsal-phalangeal and interphalangeal joints. A peculiar constriction was noted in the shafts of the proximal and distal phalanges, less marked in the metatarsal bones.

A roentgenogram of the lower thoracic spine, taken

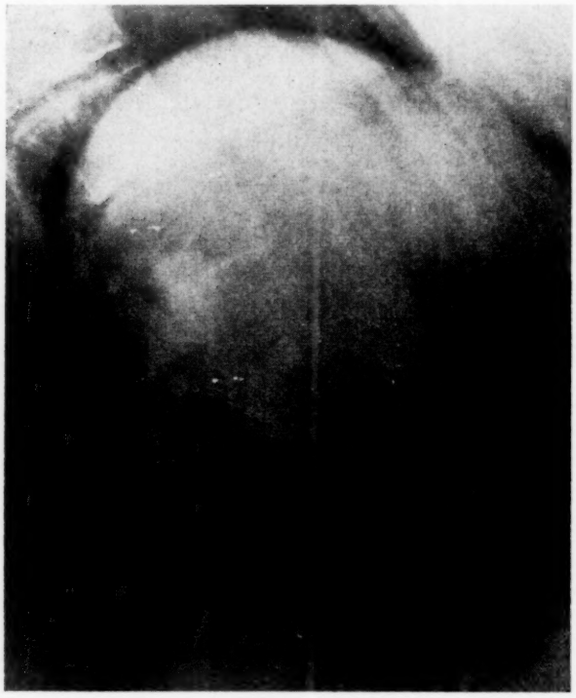


FIG. 7. Lumbar spine. Note the concavity of the dorsal surface of the vertebral bodies (arrows). Right renal staghorn calculus projected over lumbar spine.



FIG. 6. Feet. Varying degrees of phalangeal subluxations, constriction of the shafts of the phalanges and marked osteoporotic changes.

in the lateral projection, demonstrated the marked kyphosis with evidence of a decrease in the rostral-caudal diameter of several of the lower thoracic vertebral bodies, associated with osteophyte formation. Also noted was some associaetd narrowing of the intervertebral spaces probably denoting degenerative disc disease. Roentgenogram of the lumbar spine, taken in the lateral projection (Fig. 7), demonstrated an increase in the vertical diameter of the lumbar vertebral bodies. There was also evidence of an increased concavity of the posterior portions of the lumbar vertebral bodies and the lower thoracic vertebral bodies previously described in association with acromegaly [*33*]. There was an increase in the lordotic curve. Bony mineralization appeared satisfactory.

Roentgenograms of the skull in both lateral (Fig. 8) and posteroanterior projections revealed a granular appearance to the calvarium which was thought to be related to an osteoporotic process. The diploic space appeared to be thickened in the frontal and anterior parietal regions. The mastoid and ethmoid air cells were large, the frontal sinuses were huge. Cone study of the sella turcica (Fig. 9) in the lateral projection demonstrated the markedly enlarged sella turcica with evidence of erosion of the floor into the sphenoid sinus. The clinoid processes were thinned, the posterior clinoid processes displaced posteriorly. An amorphous area of calcification was noted in the posterior apsect of the sella turcica.

The hematocrit ranged between 43 and 47 per cent (average 45 per cent). Hemoglobin values ranged between 14 and 16 gm. per cent. The white cell count

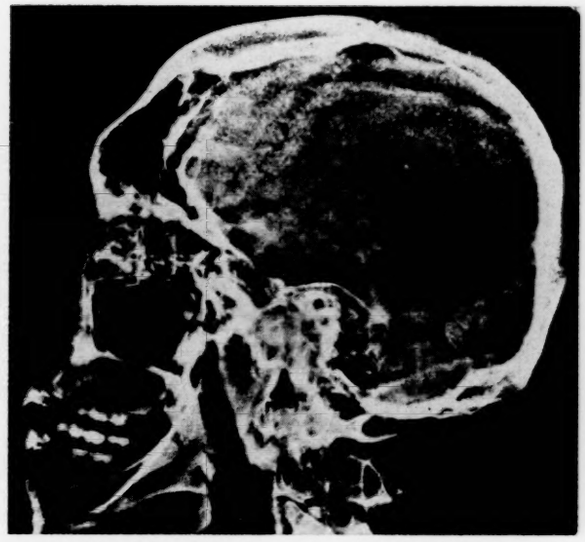


FIG. 8. Lateral projection of the skull. Note giant frontal sinuses, prominent mastoids, thickened calvarium and prognathism.



FIG. 9. Cone study of the sella turcica. Note marked enlargement of the sella turcica.

averaged 7,500 cells/cu. mm., the differential counts were always within normal limits. Eosinophil counts on fasting morning specimens ranged between 312 and 353 cells/cu. mm. After the intake of food the afternoon specimens gave eosinophil counts ranging between 153 to 210 cells/cu. mm.

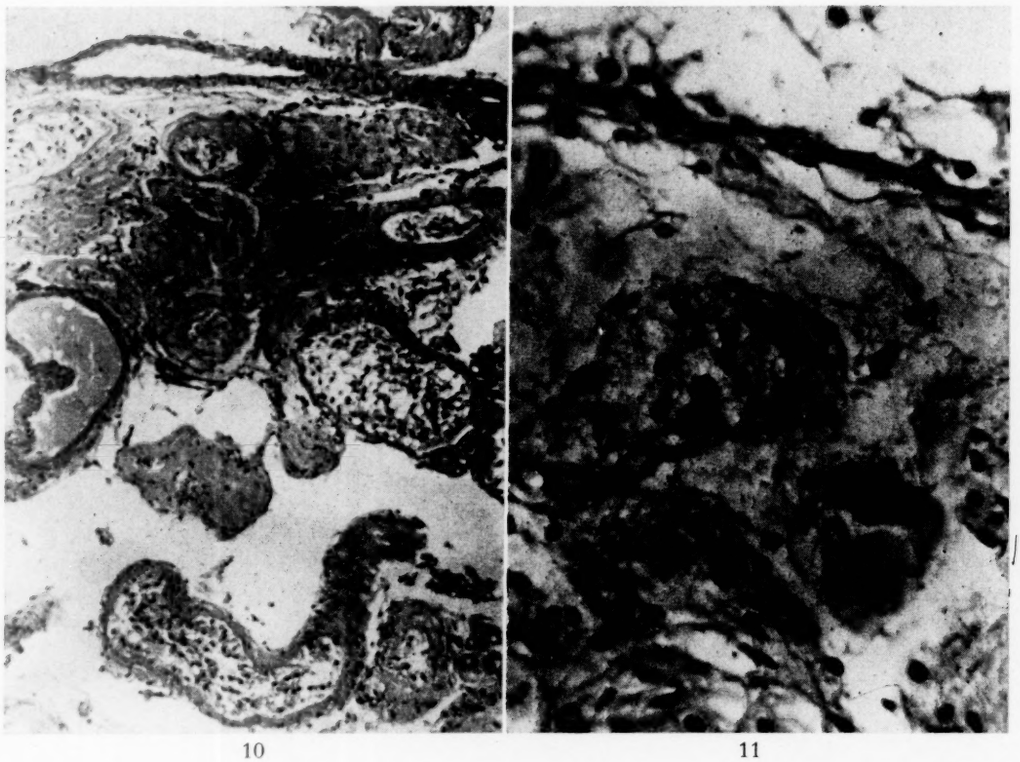
Urinalysis consistently revealed numerous white blood cells/high power field, 1 to 3 red blood cells/high power field, a trace of albumin and bacteriuria. The specific gravity ranged between 1.012 to 1.027. There was never any detectable glycosuria. Urine cultures on three occasions grew out Escherichia coli and Aerobacter aerogenes sensitive to Chloromycetin® and tetracycline. The blood urea nitrogen ranged between 12 to 16 mg. per cent. Phenolsulfonphthalein excretion was 19 per cent in fifteen minutes, 47 per cent in one hour and 61 per cent in two hours. Endogenous creatinine clearance determined in one-hour urine samples for four hours, with concomitant serum creatinine samples as measured by the Folin method, averaged .151 ml./minute, uncorrected for body surface area.

The bromsulfalein test, as measured by the colorimetric method, showed 2 per cent dye retention at the end of forty-five minutes. The one-minute Van den Bergh test showed 0.37 mg. bilirubin per cent, the thirty minute test gave 0.67 mg. per cent [*34*]. Serum total cholesterol values ranged between 190 and 220 mg. per cent [*35*]. The prothrombin time was seventeen seconds, with a control of fifteen seconds, using the modified Quick determination. The serum total protein was 8.2 gm. per cent with albumin levels of 5 gm. and globulin levels of 3.2 gm. The results of the thymol turbidity test was 2 units [*36*] and of the cephalin flocculation test 3 plus [*37*].

Serum calcium levels were never elevated above the normal range. In 1957 serum calcium levels on three occasions were 10.3, 10.5 and 10 mg. per cent. In our present study the serum calcium levels on four occasions were 9.2, 9.3, 10.3 and 10.6 mg. per cent. The serum phosphorus levels by the Fiske-Subbarow technic were consistently elevated except on one occasion, being 5, 5.1, 4.7, 4.4, 3.5, 4.8 mg. per cent. In 1957 on three occasions the serum phosphorus levels were 4.8, 4.4 and 4.2 mg. per cent.

The patient was given a 200 mg. calcium diet (2,500 calories) for four days on two separate occasions prior to determination of twenty-four–hour urine calcium and phosphorus excretion levels. The urinary calcium excretion was 374 and 233 mg./twenty-four hours. The urinary phosphorus excretion was 1,170 and 830 mg./twenty-four hours. With the exception of the 374 mg./twenty-four hours calcium determination, the remainder of these values are within the normal range for our laboratory.

Serum alkaline phosphatase levels on three occasions were 1.82, 1.92 and 1.86 Bessey-Lowrey units. Phosphate clearance and per cent tubular reabsorption of phosphorus were also determined. Clearances were obtained over one- to two-hour periods in the fasting state during the forenoon. Sufficient hydration was provided before and during urine collection to promote adequate urine output. Blood samples (drawn mid-way in the collection period) and urine specimens were measured for phosphorus by the method of Fiske and Subbarow and for creatinine by the method of Folin. Phosphate clearance and endogenous creatinine clearance were estimated by dividing minute excretion by serum concentration in milligram per milliliter. Calculations for the estimation of per cent tubular reabsorption of phosphorus were those outlined by Schaaf and Kyle [*23*]. The per cent tubular reabsorption of phosphorus was noted to be 90.3 per cent and the phosphate clearance was 14.4 ml./minute. On the evening of these studies the patient was given an infusion of calcium estimated at 10 mg./kg. of body weight (1,410 mg.) as 10 per cent calcium gluconate diluted to 500 cc. with physiologic saline



10 11

FIG. 10. Testicular biopsy specimen. Note patchy atrophy and hyalinization of seminiferous tubules.

FIG. 11. Testicular biopsy specimen. Higher magnification showing vacuolization of Leydig cells.

solution and infused over a three-hour period from 9 P.M. to midnight. The following morning repeat samples of blood and urine were taken for phosphate clearance and per cent tubular reabsorption of phosphorus. The per cent tubular reabsorption of phosphorus was noted to be 98.4 per cent and the phosphate clearance had dropped to 2.4 ml./minute. A twenty-four–hour urine collection was made to determine the urinary phosphorus excretion after calcium infusion; this was found to be 666 mg. of phosphorus in twenty-four hours.

Total twenty-four–hour urine collections for 17-ketosteroids on three occasions revealed values of 20.3, 9.8 and 6.1 mg. Twenty-four–hour urine determinations of ketogenic steroids by the Norymberski method gave values of 45 and 38 mg. Following an infusion of 25 units of aqueous ACTH in 250 cc. of physiologic saline solution over a four-hour period, repeat values disclosed a 17-ketosteroid excretion of 13.9 mg. and a ketogenic steroid excretion of 27 mg./twenty-four hours.

FSH levels measured by bio-assay technics in two separate laboratories were less than 16 mouse units in one laboratory and ± 6 mouse units in another laboratory. The basal metabolic rate was minus 32 per cent and the butanol-extractable iodine was 5.8 μg./100 cc. of serum. The result of a glucose tolerance test (using true glucose values) revealed a fasting blood sugar of 75 mg. per cent, one-half hour sample 100 mg. per cent, one hour specimen 155 mg. per cent, two hours 125 mg. per cent, three hours 60 mg. per cent, four hours 70 mg. per cent, five hours 70 mg. per cent, and a six hour sample 60 mg. per cent. A repeat glucose tolerance test on a subsequent day gave similar values. Serum amylase and lipase were within normal limits. Twenty-four–hour urine urea levels were noted to be 11.2 gm. using the diacetyl monoxime technic. The serum mucoprotein level was 18 mg. per cent in terms of galactose-mannose concentration, and following an ascorbic acid load of 400 mg. daily for one week a repeat serum mucoprotein level was recorded at 17 mg. per cent [*24*].

A testicular biopsy specimen showed patchy atrophy and fibrosis of the seminiferous tubules, the tubular structures being lined with vacuolated Sertoli cells only. (Fig. 10 and 11.) A great number of tubules showed various degrees of hyalinization. The Leydig cells were considered normal in number but atypical in appearance, with vacuolization of the cytoplasm and degenerative changes of the nuclei. Oral mucosal smears for detection of genetic sex, as outlined by Greenblatt et al. [*25*], revealed a male sex chromatin pattern. Morphologic screening of the polymorphonuclear leukocytes for sexual differentiation, as outlined by Davidson et al. [*26*], also denoted a male sex chromatin pattern.

The patient was then given radiation therapy to the pituitary gland with 250 kv. peak (H. V. I.* 2.9 copper) by means of two lateral 7 by 7 cm. ports centered over the pituitary fossa and immediately adjacent structures. The patient received 3,000 r (skin dose with back scatter) to each two lateral ports over a time interval of fifty-two days. The estimated tumor dose was 2,400 r.

The patient suffered no ill effects and there were no demonstrable subjective or objective findings on follow-up visits except for epilation over the port areas. Serial serum phosphorus determination made biweekly over a four-month period failed to demonstrate any decrease after radiation; the levels ranged from 4 to 5.4 mg. per cent.

Because of the poorly explained elevation in the 17-ketogenic steroid level it was decided to perform a Decadron® suppression test. Since the patient had received radiation therapy, new control values were obtained and the ACTH stimulation test was repeated. Two twenty-four–hour urine collections were made on two successive days for control levels. The 17-ketosteroid levels were 10.4 and 11.2 mg./twenty-four hours. The 17-ketogenic steroid levels were surprisingly lower than the control levels before radiation, being 13 and 12.6 mg./twenty-four hours. The patient was then given 80 units of ACTH (aqueous) over an eight-hour period in 500 cc. of saline infusion. A higher dose of ACTH was used because it was thought that probably the initial dose of 25 units of ACTH given before radiation was insufficient in a patient of this size. The first twenty-four–hour urine collection demonstrated a 17-ketosteroid level of 8.2 mg./twenty-four hour and a 17-ketogenic steroid level of 12.2 mg./twenty-four hours. A second twenty-four–hour urine collection immediately following the first demonstrated a 17-ketosteroid level of 16.5 mg./twenty-four hour and a 17-ketogenic steroid level of 19.8 mg./twenty-four hour.

Seven days later a four-day Decadron suppression test was begun. The patient was given 0.75 mg. Decadron orally every six hours for two days; then 3 mg. every six hours for two days. Twenty-four–hour urine specimens were collected on the second and fourth days. On the second day the urine 17-ketosteroid level was 14.8 mg./twenty-four hour and the 17-ketogenic steroid level was 15.4 mg./twenty-four hours. On the fourth day the urine 17-ketosteroid level was 11.2 mg./twenty-four hours and the 17-ketogenic steroid level was 13.6 mg./twenty-four hours.

COMMENTS

Pituitary gigantism is predominantly seen in males and very few cases have been reported in which the subjects are over 9 feet tall. McFarland [*22*] in his treatise on gigantism reported two of the thirty-one patients studied to be over 9 feet tall. All of the patients in his study were over 7 feet 6 inches in height and approximately 50 per cent were over 8 feet tall. Soffer [*2*] points out the exaggeration in reporting such cases and challenges the accuracy of some of the giants reported, especially in regard to height. Humberd [*9,10*], who has studied such cases exhaustively, concluded that probably none of the giants reported on have been much more than 9 feet tall. In the present case the patient has attained a height of 8 feet and evidence is present to suggest that the growth process is still active. Conclusive evidence in the past four years attests to the fact that he has grown 6 inches since first coming under hospital observation; moreover, serum phosphorus levels are persistently elevated.

The span is greater than the total height, and the crown to pubis length is less than the pubis to heel length, which is suggestive of eunuchoidism and not the usual finding in gigantism. However, the marked kyphoscoliosis probably contributes to some degree to the disproportion of the trunk to long bone ratio. Since secondary hypogonadism not uncommonly occurs in long-standing acromegalic gigantism, these eunuchoidal variations can also be explained on the basis of early onset of pituitary dysfunction, most likely just before or at the onset of puberty. It is suggested, therefore, that gonadal function probably never did manifest itself to any great degree at puberty and the history from the patient bears this out. The fact that the prostate was not palpable adds further weight to this possibility.

The family history in gigantism is usually that the family members are of normal height and stature. The Minneapolis giant, however, did have a grandfather who was 8 feet 4 inches in height and was known as the Norwegian giant. Although our patients' immediate family was normal, relatives on both maternal and fraternal sides were noted to be exceptionally tall. However, it must be stated that we could not find any conclusive data on the heights of those relatives, since they are all deceased and records are not available in the places where they resided.

The physical findings noted in our patient were compatible with those seen in previously reported cases, the one exception being the high arched palate noted in our patient. A review of the case histories of many of the giants

* Half value layer.

failed to disclose any notation of this except in the case of the Alton giant. This was very pronounced in our patient. The neurologic changes noted in the legs, with bilateral foot drop and peroneal atrophy, are probably secondary to peroneal nerve compression as a result of the subluxations at the knee joints.

The cardiomegaly noted on physical examination was not easy to confirm roentgenographically because of the difficulties in including the patient's chest on the ordinary x-ray film. It was the opinion of the radiologists, however, that significant cardiomegaly was not present. The electrocardiograms taken over a two-year period were interesting in that an incomplete right bundle branch block with symmetrically inverted T waves in leads III, V_1, V_2 and V_3 were noted in 1956. A repeat tracing in 1957 showed a complete right bundle branch block with inverted T waves only in leads III and V_1. Tracings during the present study again showed an incomplete right bundle branch block, marked left axis deviation and symmetrically inverted T waves in leads III, V_1, V_2 V_3 and V_4, with flat T waves in leads V_4 and V_6. Electrocardiographic findings have not been reported in giants but one would suspect that such tracings would not be unusual without intrinsic cardiac disease. Hejtmancik et al. [*27*] have reported on the cardiac findings in acromegaly. In a study of twenty-one acromegalic patients, thirteen were noted to have clinical heart disease, five had been admitted to the hospital in frank congestive heart failure. In fifteen of the twenty-one patients electrocardiograms were available. Nine of the fifteen had normal tracings, and six showed evidence of intraventricular conduction defect. Four of the six showed incomplete bundle branch block and only two had an outright left bundle branch block. Our patient had no persistent cardiovascular symptoms.

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Stimulation studies repeated four months following radiation revealed a small increase in both 17-ketosteroid and 17-ketogenic levels. This may have been related to the stimulating dose of ACTH.

Suppression studies showed no change in 17-ketosteroid or 17-ketogenic steroid levels which, despite the fact that the control levels were not elevated, still does not rule out an adrenal tumor or hyperplasia.

Serum calcium levels are usually normal in acromegaly and gigantism, and such was the case in this patient. Serum phosphorus levels have not been recorded in gigantism but would presumably be elevated in view of the pituitary overactivity, just as in acromegaly. Our patient had persistently elevated serum phosphorus levels, which is highly suggestive of pituitary hyperfunction, which we believe is still present to some degree. Serum alkaline phosphatase levels are not available in gigantism but Hurxthal [*27*] has suggested that they would be increased in the active state of the disease. Acromegalic subjects usually have normal serum alkaline phosphatase levels and in our patient this was the case.

Phosphate clearance values in normal subjects, according to Kyle, Schaaf and Canary [*32*], range between 6.3 and 15.5 ml./minute, the mean values being 10.8 to 12.7 ml./minute. On the control day, our patient had a phosphate clearance of 14.4 ml./minute. However, on the day after the night-time infusion of calcium, the phosphate clearance dropped to an extremely low level of 2.4 ml./minute. Correspondingly, the total twenty-four–hour urine total phosphorus dropped from a control level of 1,170 to 660 mg./twenty-four hours. This would suggest that a normal parathyroid regulatory mechanism was present.

The per cent tubular reabsorption of phosphorus in normal subjects has been recorded at 91.3 $\pm$ 3.3 per cent [*23*]. On the control day, our patient had a value of 90.3 per cent tubular reabsorption of phosphorus, and on the day after the calcium infusion the per cent tubular reabsorption of phosphorus had risen to 98.4. Although it is difficult to interpret values in the ninetieth percentile range, this is again suggestive of a normal parathyroid regulatory mechanism, with increased phosphorus retention in the face of an exogenous calcium load. To the best of our knowledge such studies have not been previously reported in gigantism.

The phenol red test in our patient showed a diminution of dye excretion in all timed specimens, which is in keeping with the clinical findings of pyelonephritis and renal calculus in the right calyceal system. The endogenous creatinine clearance values were normal, in the range of 151 to 153 ml./minute. Gershberg et al. [*1*] found that the glomerular filtration rate and

proximal tubular activities were markedly increased in an acromegalic giant, reflecting the increased size of the glomeruli and proximal tubules, as verified at postmortem examination.

SUMMARY

A case of acromegalic gigantism is reported in a twenty-seven year old man who had not been treated prior to this study. Various laboratory studies were made to evaluate specific organ functions, especially from the endocrinologic viewpoint.

A brief comparison is made with other previously reported cases.

Acknowledgment: We are indebted to the Radiology Department and the Department of Clinical Pathology, Mercy Hospital, for their untiring cooperation in performing the various studies. We are also most appreciative of the advice and guidance given by John J. Canary, M.D., Chief of Endocrinology and Metabolism, Georgetown University Medical Center, Washington, D. C.

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Sarcoidosis of the Central Nervous System*

Report of a Case Simulating Intracranial Neoplasm

J. Robert Thompson, M.D.

Chicago, Illinois

Sarcoidosis involving the nervous system is not rare but few cases with cerebral involvement have been recorded. A review by Colover [*1*] in 1948 of the records of 115 patients with nervous system involvement revealed that only a few had brain or spinal cord lesions. These few patients had focal or generalized convulsions and other localizing signs and symptoms. Pennell [*2*] reviewed fifty-one case reports of sarcoidosis of the central nervous system and found that eight patients experienced one or more generalized seizures at some time during their illness. Another interesting observation was a low sugar content in the spinal fluid which suggested the possibility that this granulomatous lesion represented an infectious process. A few years later (1952) Gendel and his coworkers [*3*] reviewed twenty-four additional case reports and found that the central nervous system was involved in eight cases. The structures most commonly involved were the facial nerves.

CASE REPORT

The patient was a forty-four year old Negro woman admitted to the Municipal Tuberculosis Sanitarium on October 8, 1958. She had been well until March 1955 when it was thought that she had suffered a slight stroke because of complaints of numbness of the left side of the face, headache, weakness and vertigo. Bed rest and medication were prescribed, but after a period of several months she had lost considerable weight and strength. In July 1958 she experienced episodes of paroxysmal nocturnal dyspnea and complained of marked dyspnea on exertion. A chest roentgenogram was requested by her physician because of "fluid on her lung" and it showed an infiltration in the base of the right lung.

This patient worked as a poultry pinner for five years, and in a box factory two years prior to that. Past illnesses included measles and pertussis in childhood. Her mother had diabetes mellitus and one brother had high blood pressure.

On admission to the Sanitarium, examination revealed a fairly well nourished Negro woman with a blood pressure of 160 mm. Hg systolic and 110 mm. Hg diastolic. Dullness to percussion was noted from the fifth rib down to the base of the right side posteriorly and the breath sounds were diminished over the base of the right lung. There was slight numbness of the left cheek. The remainder of the examination was within normal limits.

A hemogram taken on admission showed a leukocyte count of 4,300 per cu. mm. with a differential count of 60 per cent neutrophils, 20 per cent lymphocytes and 11 per cent monocytes. The erythrocyte count was 4,590,000 per cu. mm. and the hemoglobin level was 13 gm. per 100 ml. The fasting blood sugar was 205 mg. per 100 ml.; the non-protein nitrogen was 40 mg. per 100 ml. The serum albumin was 4.1 gm., globulin 4.6 gm. and gamma globulin 2.96 gm. per 100 ml. The urinalysis was normal and the result of the serologic test for syphilis was negative. The reaction to the tuberculin test was positive in all dilutions.

A chest roentgenogram showed a soft tissue prominence in the region of the right paratracheal node group. An extensive pleural effusion obscured the lower portion of the right lung field. Many sputum and gastric lavage specimens were negative for acid-fast bacilli and for fungi. The pleural fluid was negative for acid-fast bacilli and the cell count was 580 lymphocytes per cu. mm.

The patient was given antimicrobial therapy including streptomycin, isoniazid and para-aminosalicylate. A glucose tolerance test revealed a mild diabetic curve; the patient's diabetes was controlled by diet alone. There was some relief of symptoms after a period of bed rest and supportive therapy, but approximately two months after admission she was found unconscious on the wash room floor. There were intermittent generalized convulsive twitchings especially noticeable on the left side of the face. The patient was speechless and semiconscious for about one week and after four weeks was ambulatory. A second seizure,

* From the City of Chicago, Municipal Tuberculosis Sanitarium, Chicago, Illinois. Manuscript received November 24, 1960.

failed to disclose any notation of this except in the case of the Alton giant. This was very pronounced in our patient. The neurologic changes noted in the legs, with bilateral foot drop and peroneal atrophy, are probably secondary to peroneal nerve compression as a result of the subluxations at the knee joints.

The cardiomegaly noted on physical examination was not easy to confirm roentgenographically because of the difficulties in including the patient's chest on the ordinary x-ray film. It was the opinion of the radiologists, however, that significant cardiomegaly was not present. The electrocardiograms taken over a two-year period were interesting in that an incomplete right bundle branch block with symmetrically inverted T waves in leads III, V_1, V_2 and V_3 were noted in 1956. A repeat tracing in 1957 showed a complete right bundle branch block with inverted T waves only in leads III and V_1. Tracings during the present study again showed an incomplete right bundle branch block, marked left axis deviation and symmetrically inverted T waves in leads III, V_1, V_2 V_3 and V_4, with flat T waves in leads V_4 and V_6. Electrocardiographic findings have not been reported in giants but one would suspect that such tracings would not be unusual without intrinsic cardiac disease. Hejtmancik et al. [*27*] have reported on the cardiac findings in acromegaly. In a study of twenty-one acromegalic patients, thirteen were noted to have clinical heart disease, five had been admitted to the hospital in frank congestive heart failure. In fifteen of the twenty-one patients electrocardiograms were available. Nine of the fifteen had normal tracings, and six showed evidence of intraventricular conduction defect. Four of the six showed incomplete bundle branch block and only two had an outright left bundle branch block. Our patient had no persistent cardiovascular symptoms.

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SUMMARY

A case of acromegalic gigantism is reported in a twenty-seven year old man who had not been treated prior to this study. Various laboratory studies were made to evaluate specific organ functions, especially from the endocrinologic viewpoint.

A brief comparison is made with other previously reported cases.

Acknowledgment: We are indebted to the Radiology Department and the Department of Clinical Pathology, Mercy Hospital, for their untiring cooperation in performing the various studies. We are also most appreciative of the advice and guidance given by John J. Canary, M.D., Chief of Endocrinology and Metabolism, Georgetown University Medical Center, Washington, D. C.

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Sarcoidosis of the Central Nervous System*

Report of a Case Simulating Intracranial Neoplasm

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Sarcoidosis involving the nervous system is not rare but few cases with cerebral involvement have been recorded. A review by Colover [*1*] in 1948 of the records of 115 patients with nervous system involvement revealed that only a few had brain or spinal cord lesions. These few patients had focal or generalized convulsions and other localizing signs and symptoms. Pennell [*2*] reviewed fifty-one case reports of sarcoidosis of the central nervous system and found that eight patients experienced one or more generalized seizures at some time during their illness. Another interesting observation was a low sugar content in the spinal fluid which suggested the possibility that this granulomatous lesion represented an infectious process. A few years later (1952) Gendel and his coworkers [*3*] reviewed twenty-four additional case reports and found that the central nervous system was involved in eight cases. The structures most commonly involved were the facial nerves.

CASE REPORT

The patient was a forty-four year old Negro woman admitted to the Municipal Tuberculosis Sanitarium on October 8, 1958. She had been well until March 1955 when it was thought that she had suffered a slight stroke because of complaints of numbness of the left side of the face, headache, weakness and vertigo. Bed rest and medication were prescribed, but after a period of several months she had lost considerable weight and strength. In July 1958 she experienced episodes of paroxysmal nocturnal dyspnea and complained of marked dyspnea on exertion. A chest roentgenogram was requested by her physician because of "fluid on her lung" and it showed an infiltration in the base of the right lung.

This patient worked as a poultry pinner for five years, and in a box factory two years prior to that. Past illnesses included measles and pertussis in childhood. Her mother had diabetes mellitus and one brother had high blood pressure.

On admission to the Sanitarium, examination revealed a fairly well nourished Negro woman with a blood pressure of 160 mm. Hg systolic and 110 mm. Hg diastolic. Dullness to percussion was noted from the fifth rib down to the base of the right side posteriorly and the breath sounds were diminished over the base of the right lung. There was slight numbness of the left cheek. The remainder of the examination was within normal limits.

A hemogram taken on admission showed a leukocyte count of 4,300 per cu. mm. with a differential count of 60 per cent neutrophils, 20 per cent lymphocytes and 11 per cent monocytes. The erythrocyte count was 4,590,000 per cu. mm. and the hemoglobin level was 13 gm. per 100 ml. The fasting blood sugar was 205 mg. per 100 ml.; the non-protein nitrogen was 40 mg. per 100 ml. The serum albumin was 4.1 gm., globulin 4.6 gm. and gamma globulin 2.96 gm. per 100 ml. The urinalysis was normal and the result of the serologic test for syphilis was negative. The rea tion to the tuberculin test was positive in all dilutions.

A chest roentgenogram showed a soft tissue prominence in the region of the right paratracheal node group. An extensive pleural effusion obscured the lower portion of the right lung field. Many sputum and gastric lavage specimens were negative for acid-fast bacilli and for fungi. The pleural fluid was negative for acid-fast bacilli and the cell count was 580 lymphocytes per cu. mm.

The patient was given antimicrobial therapy including streptomycin, isoniazid and para-aminosalicylate. A glucose tolerance test revealed a mild diabetic curve; the patient's diabetes was controlled by diet alone. There was some relief of symptoms after a period of bed rest and supportive therapy, but approximately two months after admission she was found unconscious on the wash room floor. There were intermittent generalized convulsive twitchings especially noticeable on the left side of the face. The patient was speechless and semiconscious for about one week and after four weeks was ambulatory. A second seizure,

* From the City of Chicago, Municipal Tuberculosis Sanitarium, Chicago, Illinois. Manuscript received November 24, 1960.

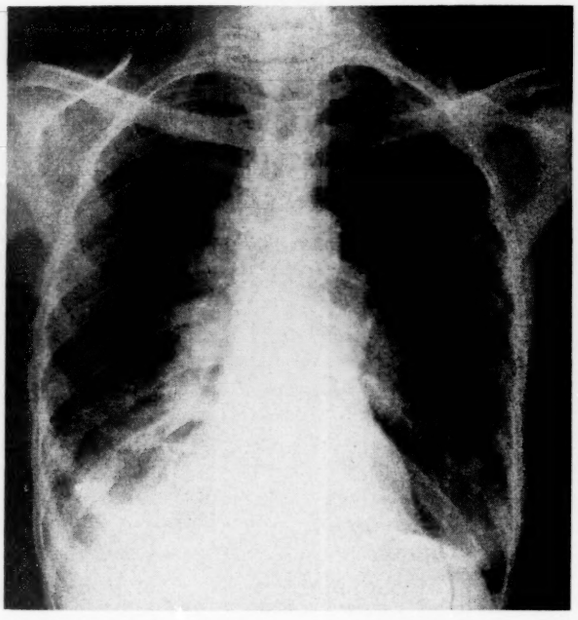


FIG. 1. Chest roentgenogram taken approximately one month before the patient died. Note the prominent hilar regions.

of lesser severity and without loss of consciousness, occurred one week later. Following these episodes the patient was transferred to a general hospital for neurologic evaluation. Angiograms and cerebral air studies were reported as normal. The patient was transferred back to the Sanitarium in July 1959 with a diagnosis of cerebrovascular lesion of undetermined type with encephalomalacia. The chest roentgenogram (Fig. 1) at this time still showed a soft tissue prominence in the hilum, but there was considerable clearing of the lower right lung field. Scalene lymph nodes taken for biopsy were reported as showing reactive hyperplasia. Blood chemistry values were comparable to those reported at the time of admission.

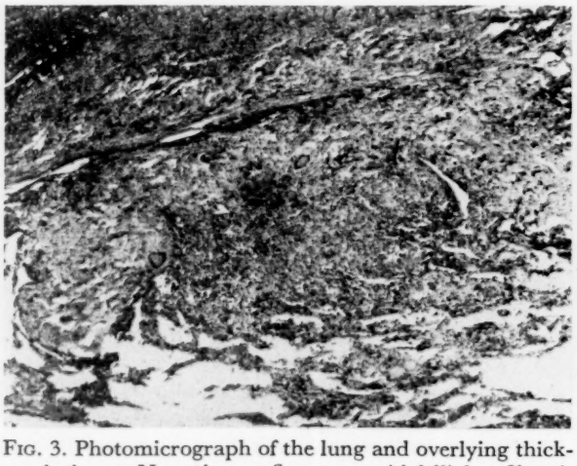


FIG. 3. Photomicrograph of the lung and overlying thickened pleura. Note the confluent sarcoid follicles, fibrosis and thickened alveolar septa.

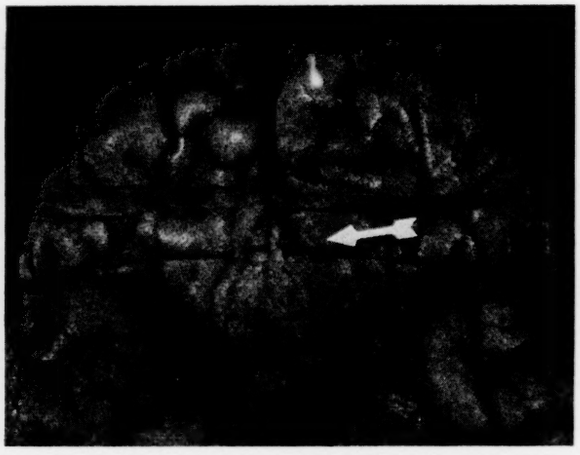


FIG. 2. A photograph of the brain showing the inferior portion of the organ. Note how the left olfactory tract is embedded in the surrounding tissue and is barely discernable as a nerve structure (arrow). The meninges over the left side are somewhat thickened and opaque.

The patient was given Dilantin® to help control the seizures, but the attacks recurred with increasing frequency and in August 1959 she became comatose and died.

At autopsy the significant changes were in the lungs and brain. (Fig. 2.) The right lung was adherent to the chest wall by fibrinous and fibrous adhesions. The visceral pleura was thickened, especially over the lower lobe of the right lung. There were antemortem thrombi in the branches of the pulmonary artery and several firm areas were palpable in either lung. All the lymph nodes were enlarged and soft to rubbery in consistency. The cut surfaces of the lungs showed a fine reticular fibrosis with many small dark red to purple-red firm areas measuring up to 1 cm. in greatest dimension. The brain appeared symmetrical, but in the process of removing the organ it was found to be adherent to the base of the skull in the region

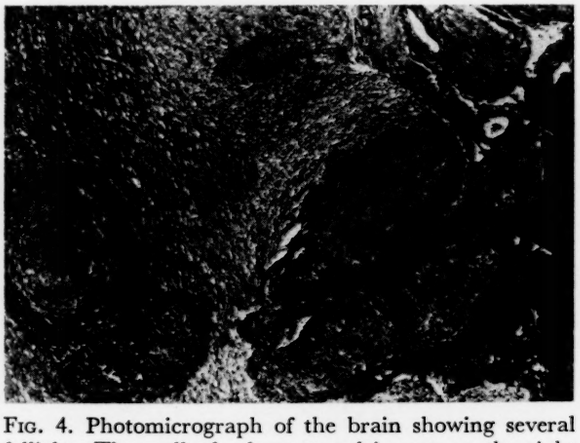


FIG. 4. Photomicrograph of the brain showing several follicles. The wall of a large vessel is seen on the right margin of the picture. The centers of two follicles are composed of a hyalinized substance.

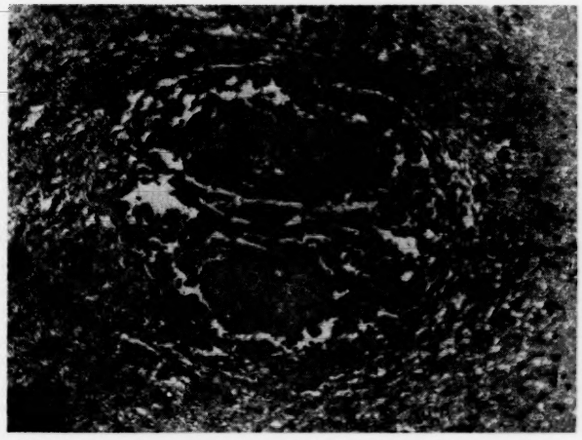


FIG. 5. A solitary follicle which has been converted to a mass of hyalinized material with a paucity of epithelioid cells.

of the inferior portion of the left frontal lobe. The olfactory nerve on this side was imbedded in firm pale yellow-tan tissue which was poorly demarcated from the cerebral cortex. The pelvic veins contained antemortem blood clots.

Microscopic examination showed a diffuse fibrosis of the lungs with dense hyalinized scar tissue in some areas, especially the subpleural region and about the branch vessels and bronchi. There were ill defined to confluent active sarcoid follicles (Fig. 3) composed of epithelioid cells and multinucleated giant cells. Some of the giant cells contained inclusion bodies which, for the most part, were poorly staining anisotropic fragments. There was dilatation of the bronchi and bronchioles, and cystic dilatation of the air spaces. Sections of the brain tissue showed many discrete to confluent follicles (Fig. 4) in the meninges and within the cortex. Many of these follicles represented active sarcoid lesions and others were completely hyalinized. (Fig. 5.) In some instances the lesions were located about the vessels of the meninges but, in addition, they occurred as solitary lesions within the brain tissue. Sections of lymph nodes draining the lungs showed almost complete replacement by confluent sarcoid lesions. There were many multinucleated giant cells present, some of which contained poorly staining anisotropic inclusion bodies. A few conchoid bodies were seen. There was considerable para-amyloid material deposited in the nodes. Sections stained with periodic acid-Schiff (PAS) and acid-fast stains revealed the inclusion bodies to be partially acid-fast and partially PAS-positive. As far as could be determined by serial sections, the same material stained positively in both instances. Another feature of the lymph node sections was involvement of vessel walls with active sarcoid lesions. It is difficult to say whether or not this represents primary involvement of the wall of the vascular space or encroachment on the wall of the vascular space by an expanding lesion. At any rate, in many instances one saw follicles projecting into vascular spaces and partially covered by endothelium. A final histologic feature was the presence of many chronic inflammatory cells in the myocardium of the left ventricle. There was some atrophy of muscle fibers but little fibroblastic response. This was interpreted as a chronic myocarditis of non-specific nature.

COMMENT

After reviewing the literature one is impressed with the fact that the diagnosis of sarcoidosis of the central nervous system is seldom made without the benefit of histologic examination of tissue removed at the time of craniotomy or laminectomy [*4*]. As a consequence of the failure to establish a definitive diagnosis early in the course of the disease, only a few patients have had the benefit of steroid therapy. There is a possibility that if the administration of steroids was commenced early in the course of the disease there would be some degree of involution and hence a more favorable prognosis [*5*]. Wood and Bream [*6*] expressed the view that the effects of the lesions in the central nervous system were mechanical rather than toxic. It is my opinion that lung involvement precedes extrapulmonary sarcoidosis in the majority of cases, and establishment of the diagnosis of lung sarcoidosis and treatment at an early stage would most likely prevent an extension of the disease to other organ systems. This prompts the question, how does sarcoid reach the central nervous system? The notion that this represents a vascular dissemination [*7*] has been proposed. In this case, there were sarcoid lesions in the lymph nodes involving the walls of the vascular spaces with extension into the lumens. Michaels and his co-workers [*8*] recently described arterial changes in three cases of pulmonary sarcoidosis. These consisted of giant cell granulomatous lesions in the branches of the pulmonary artery, with intimal thickening and rupture of the internal elastic lamina. They were of the opinion that these lesions suggested a relationship between sarcoidosis and polyarteritis nodosa. These findings tend to support the notion that there is vascular dissemination of sarcoid lesions to other organ systems.

Histologically the lesions were similar to the classic picture described many years ago. The most active sarcoid follicles were noted in the lymph nodes draining the lungs; there was complete lack of caseation necrosis with considerable deposition of para-amyloid material. The sarcoid

lesions in the brain were similar save for some areas in which there was cuffing of the follicles by small dark-staining cells. The inclusions noted in the large multinucleated giant cells were principally of the anisotropic type and, interestingly enough, were partially stained by acid-fast and PAS stains. No structures resembling tubercle bacilli were present in any of the sections.

The fact that the patient had a pleural effusion of some months' standing is interesting inasmuch as pleural effusion is not usually associated with sarcoidosis. The incidental finding of a marked degree of chronic myocarditis cannot be associated in any way with the sarcoidosis, but the myocarditis was undoubtedly a factor in her death. There was no evidence of any right-sided heart enlargement on the basis of pulmonary obstruction. The patient was known to have had essential hypertension for several years.

SUMMARY

A case of sarcoidosis involving the central nervous system is described. This is considered to be a complication of pulmonary sarcoidosis, present in the patient for a period of at least one year as demonstrated by chest roentgenograms. The patient had symptoms simulating an intracranial neoplasm, but because of the presence of a cardiac disorder this was interpreted as cerebrovascular disease.

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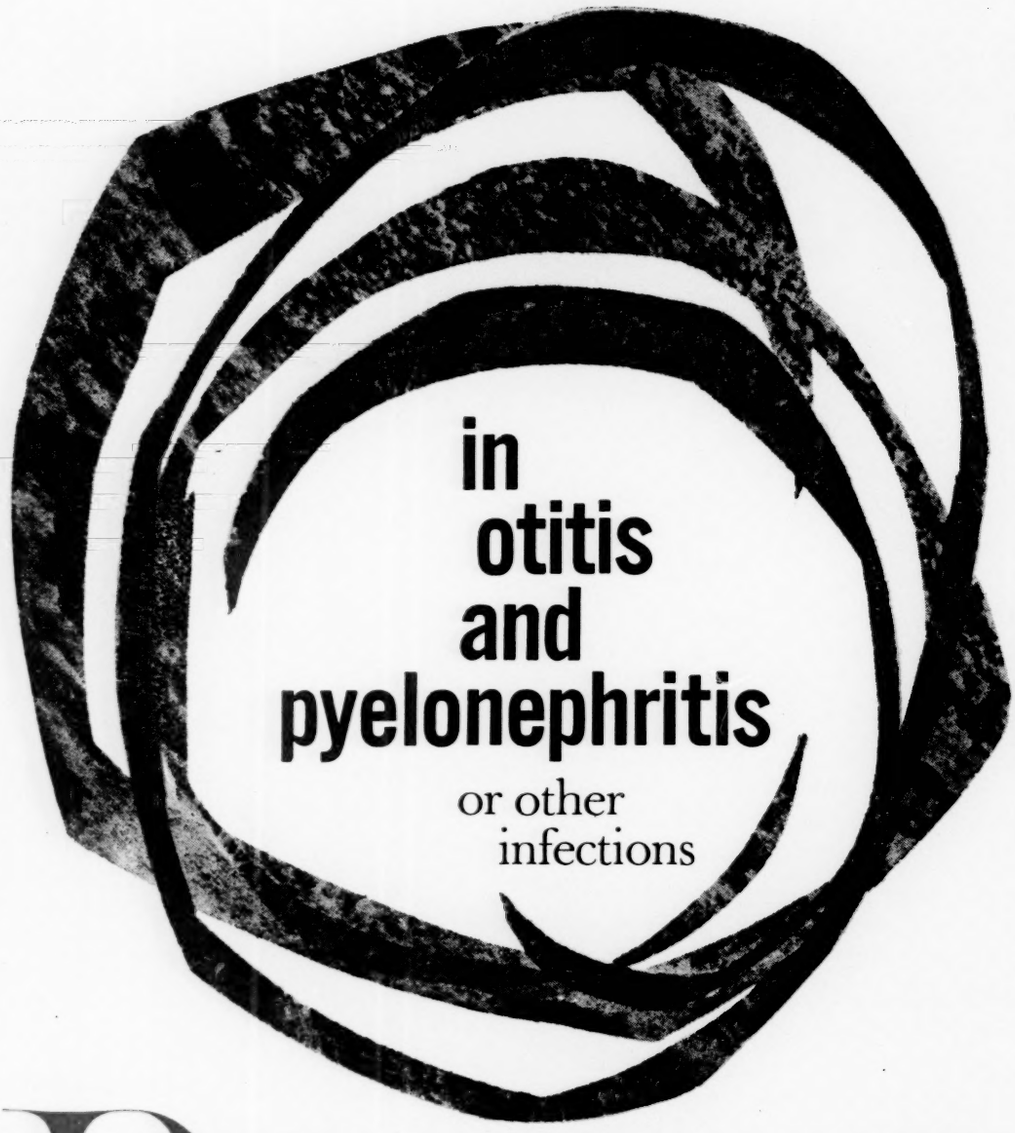
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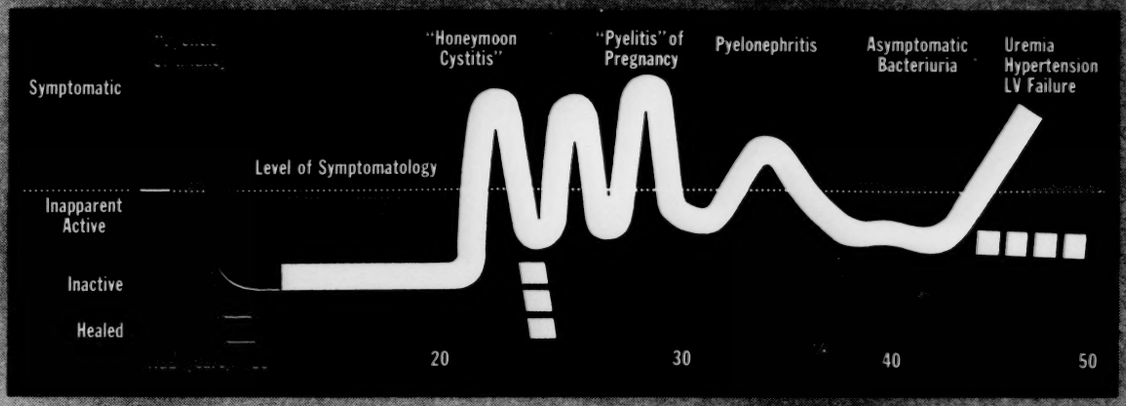
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REFERENCES: 1. Birchall, R.: Am. Practit. *11*:918, 1960. 2. Stevenson, S. S.: J. Louisiana Med. Soc. *110*:219, 1958. 3. Marshall, M., Jr.: J. Kentucky Med. Assoc. *59*:35, 1961. 4. Johnson, S. H., III, and Marshall, M., Jr.: J. Urol. *82*:162, 1959.

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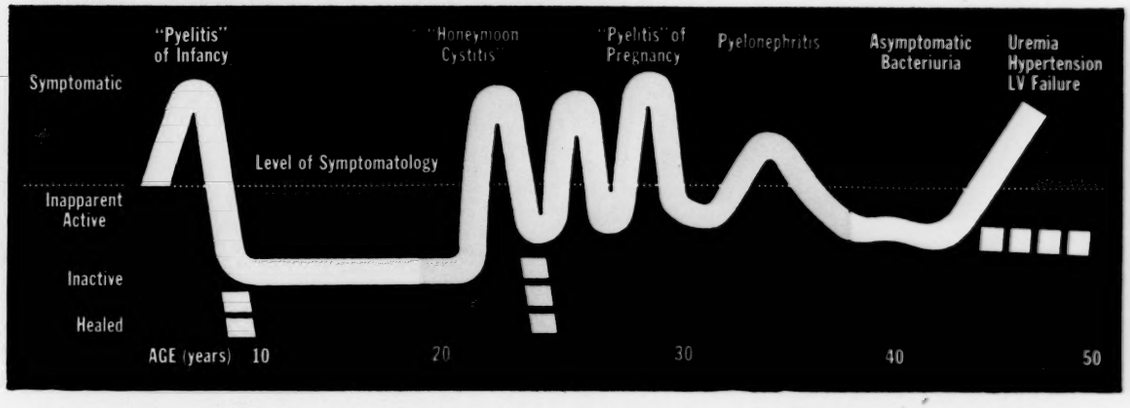
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REFERENCES: 1. Birchall, R.: Am. Practit. *11*:918, 1960. 2. Benson, R. C., and Mitchell, J. C.: Clin. Obstet. Gynec. *1*:97, 1958. 3. Favour, C. B.: Southern Med. J. *54*:848, 1961. 4. Nesbitt, R. E. L., Jr., and Young, J. E.: Obstet. Gynec. (N. Y.) *10*:89, 1957.

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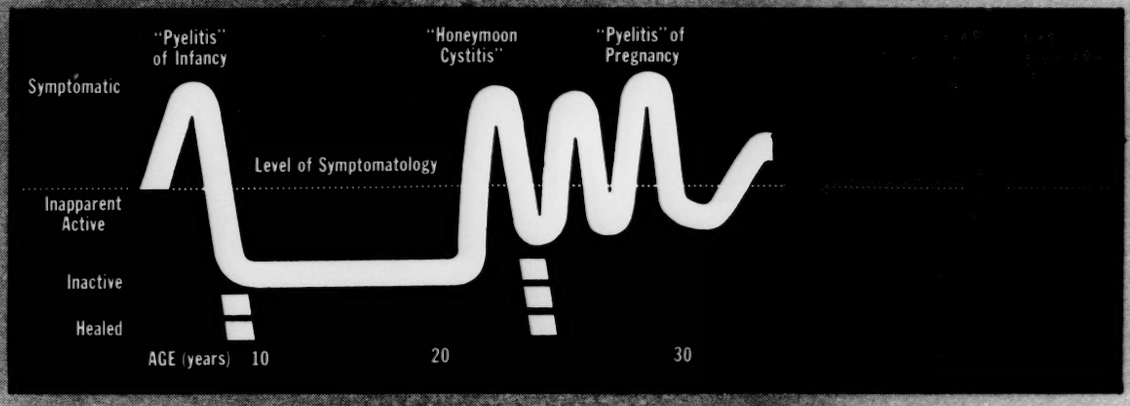
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REFERENCES: 1. Birchall, R.: Am. Practit. *11*:913, 1960. 2. Jawetz, E., et al.: A.M.A. Arch. Intern. Med. *100*:549, 1957. 3. Lippman, R. W., et al.: J. Urol. *80*:77, 1958.

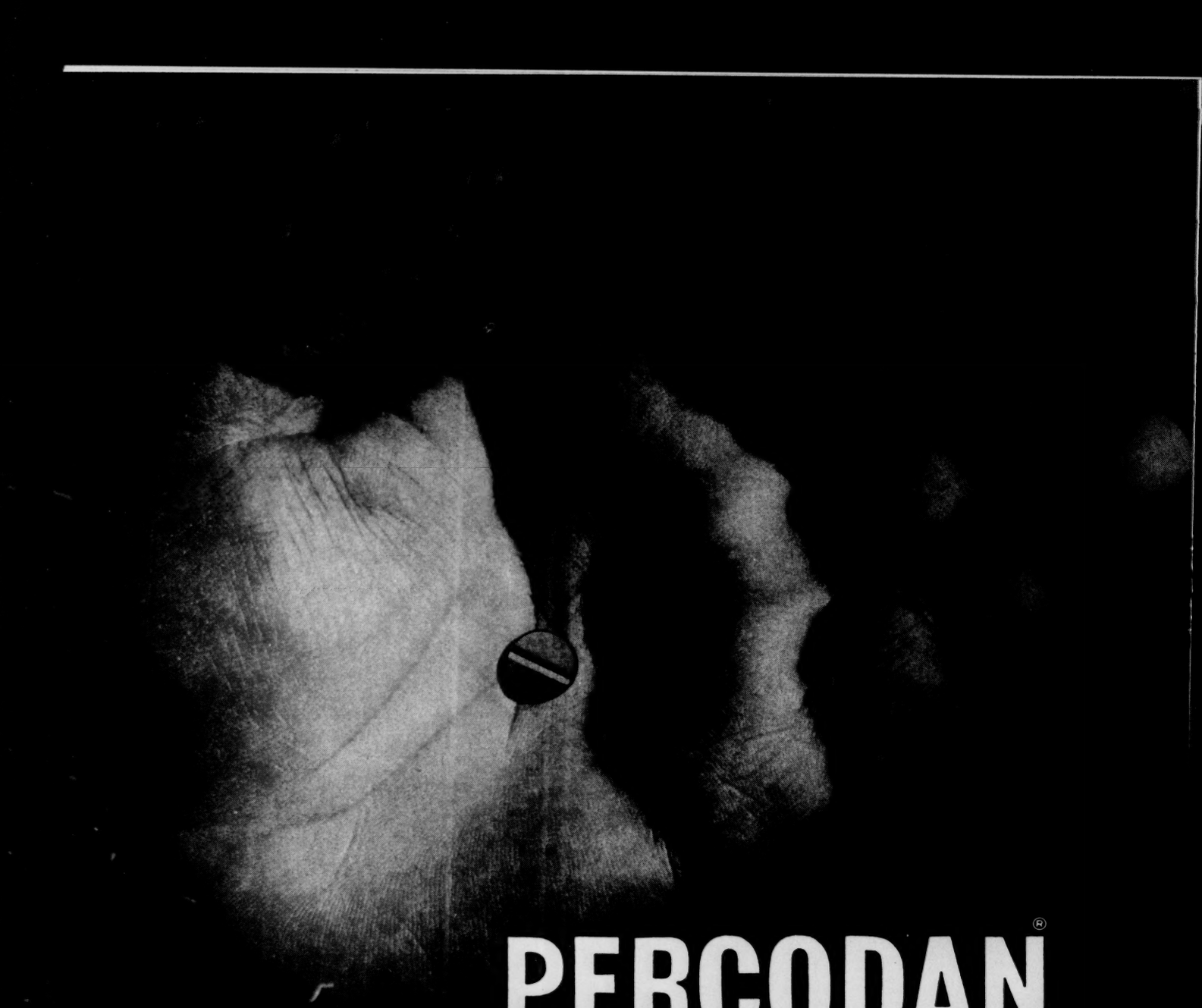
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ADMINISTRATION AND DOSAGE: Dosage varies with the state and response of each patient, rather than with weight and should be individualized by the physician for optimum results. *Recommended oral dosage:* In anxiety and tension states, senility, alcoholism, pre- and postoperative and pre- and postpartum tension and emesis: up to 400 mg. daily in divided doses. In anxiety associated with asthma, neurodermatoses, menopausal syndrome, digestive disorders, functional or essential hypertension, tension headaches: 50 mg. q.i.d. initially—adjust according to response. In cardiac arrhythmias: initial—25 mg. q. 6 h. until arrhythmia disappears; maintenance or prophylactic—50-75 mg. daily in divided doses. In pediatric behavior problems under 6 years: 50 mg. daily in divided doses. Six and over: 50-100 mg. daily in divided doses. *Recommended parenteral dosage:* In preoperative, obstetrical, and more emergent situations in other indications: 25-100 mg. I.M. or I.V. q. 4 h., p.r.n. In cardiac arrhythmias: 50-100 mg. I.M. stat, and q. 4-6 h., p.r.n.; maintain with 25 mg. b.i.d. or t.i.d.

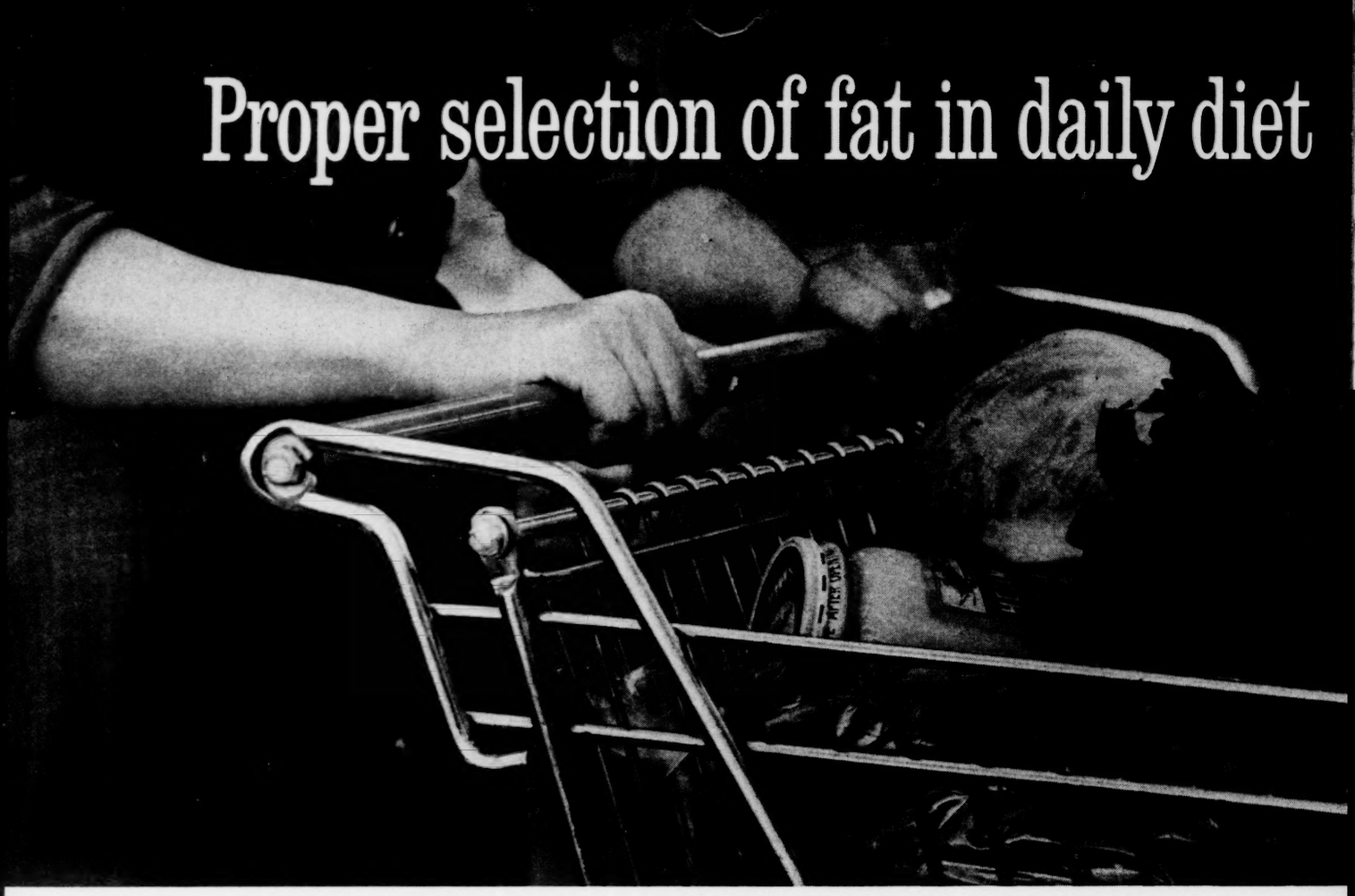
SIDE EFFECTS: Drowsiness may occur in some patients; if so, it is usually transitory, disappearing within a few days of continued therapy or upon reduction of dosage. Dryness of mouth may be encountered at higher doses.

PRECAUTIONS: The potentiating action of hydroxyzine should be taken into account when the drug is used in conjunction with central nervous system depressants. Do not exceed 1 cc. per minute I.V. Do not give over 100 mg. per dose I.V. Parenteral therapy is usually for 24-48 hours, except when, in the judgement of the physician, longer-term therapy by this route is desirable.

SUPPLIED: VISTARIL *Capsules* (hydroxyzine pamoate)—25, 50, and 100 mg. VISTARIL *Oral Suspension* (hydroxyzine pamoate)—25 mg. per 5 cc. teaspoonful. VISTARIL *Parenteral Solution* (hydroxyzine hydrochloride)—10 cc. vials, 25 mg. per cc.; 2 cc. ampules, 50 mg. per cc.

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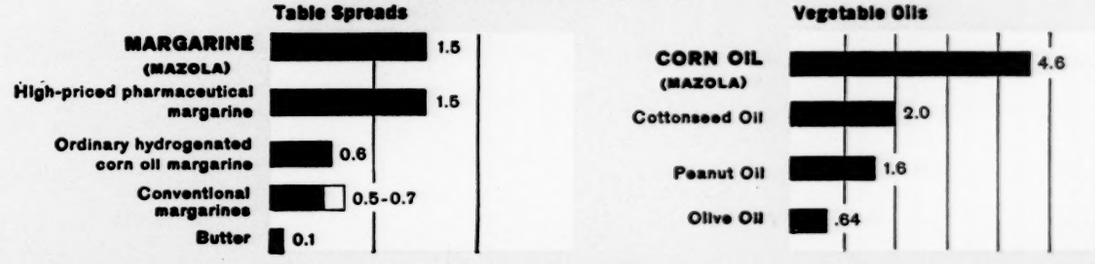
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Fatty Acids				
Polyunsaturated	21	12	51	14
Monounsaturated	40	23	32	9
Saturated	14	8	11	3
Natural Sitosterols	0.5	0.3	1	0.3
Natural Tocopherols	0.08	0.045	0.1	0.03
Cholesterol	none	none	none	none
Sodium	0.9	0.5	none	none

MAZOLA MARGARINE—410 Calories/2 oz.; Iodine Value—96
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(1) Carter, S.: *M. Clin. North America* **37**:315, 1953. (2) Maltby, G. L.: *J. Maine M. A.* **48**:257, 1957. (3) Thomas, M. H., in Green, J. R., & Steelman, H. F.: Epileptic Seizures, Baltimore, The Williams & Wilkins Company, 1956, p. 43.

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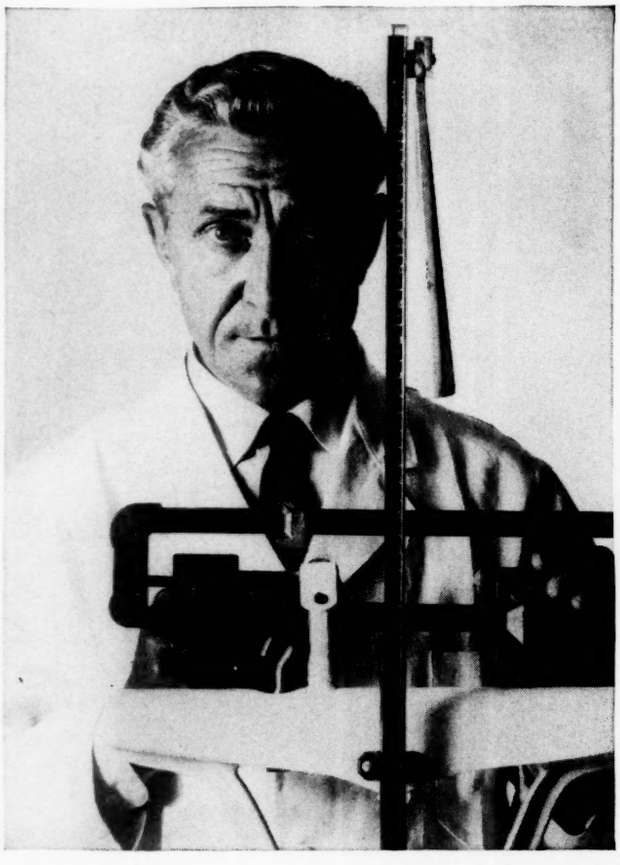
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See page 51 for description, indications, dosage, precautions, side effects, and how supplied.

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INDICATIONS: RENESE is indicated for the treatment of hypertension and edema. It has been found useful in congestive heart failure, fluid retention of pregnancy, premenstrual tension, obesity (where fluid retention is present), renal edema, cirrhosis, drug-induced edema, and toxemia of pregnancy.

ADMINISTRATION AND DOSAGE: *Initial dose:* Depending on the severity of the conditions, initial doses of RENESE may range from 1 mg. to 4 mg. daily (refractory cases may require as much as 12 mg. daily). *Maintenance dose:* Usual effective maintenance doses range from 1 mg. to 4 mg. daily, depending on the severity of the cases. Some patients have responded to 1 mg. every other day (0.5 mg. daily).

SIDE EFFECTS AND PRECAUTIONS: Since all diuretic agents may reduce serum levels of sodium, chloride, and potassium, patients on RENESE should be observed regularly for early signs of fluid or electrolyte imbalance. Caution must be exercised during digitalis administration to prevent hypokalemia since patients are then more sensitive to the development of digitalis toxicity. During RENESE therapy of edema in patients with chronic renal disease, routine precautions should be taken against renal failure as indicated by an increasing blood urea nitrogen. Like other thiazide diuretics, RENESE may cause a rise in serum uric acid levels and should therefore be used with caution in patients with gout. Should overt manifestations of gout appear, the concomitant use of uricosuric agents may be effective in relieving the symptoms. Side effects with RENESE, such as nausea, vertigo, weakness, and fatigue are infrequent and seldom require cessation of therapy. Most of these reactions may be overcome by reducing the dose of RENESE or by taking measures to improve any electrolyte imbalance. Mild maculopapular skin rash has been rarely reported. Extra precautions may be necessary in patients who may require norepinephrine, or curare or its derivatives.

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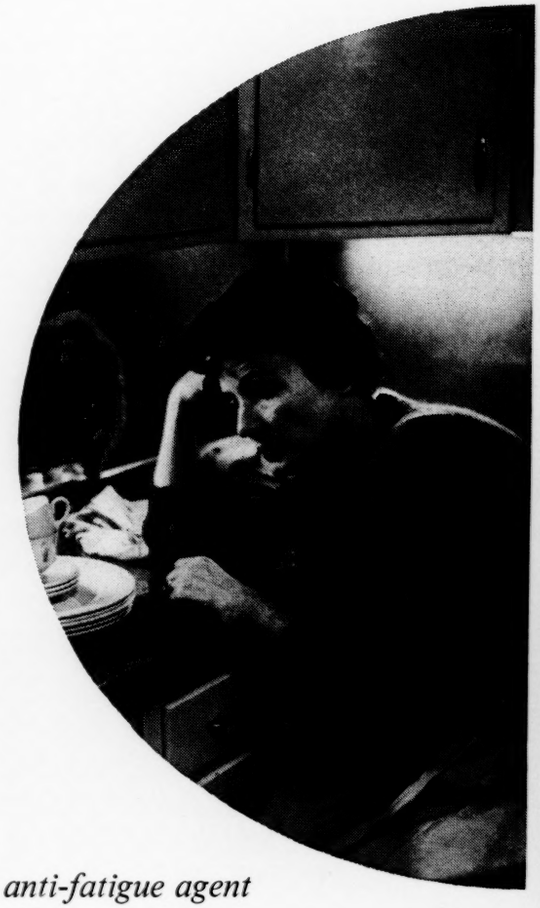
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1. Ford, R. V.: Current Therap. Res. 3:320, July, 1961.

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For further information on limitations, administration, and prescribing of SPARTASE see descriptive literature or current Direction Circular.

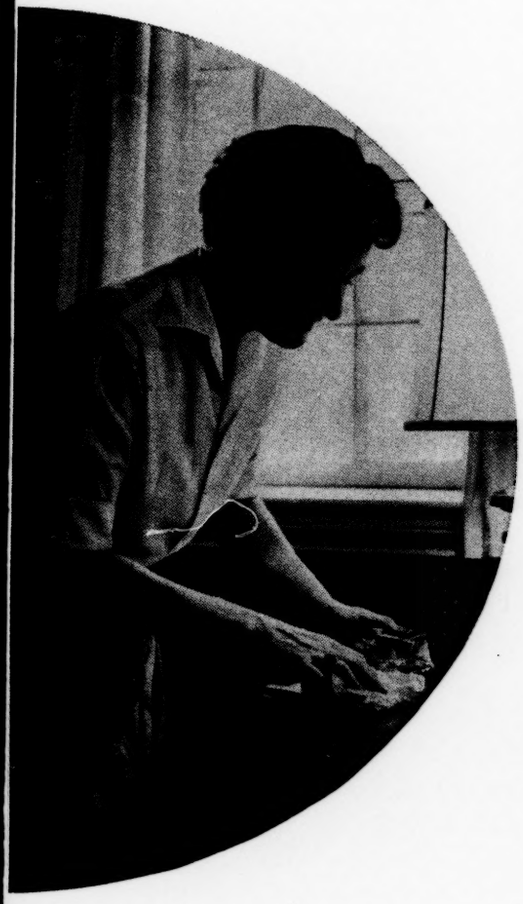
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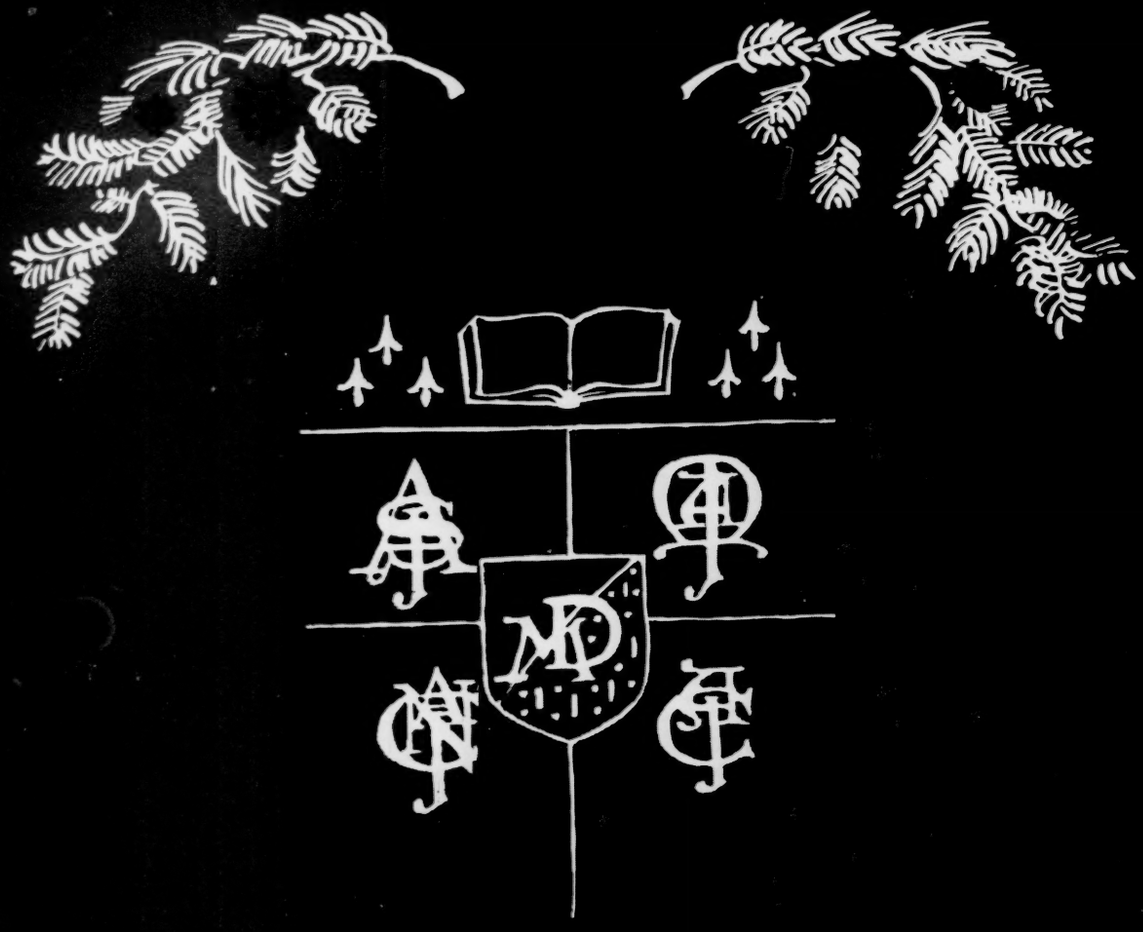
fatigue accompanying organic conditions[1-3] —such as postinfluenzal syndrome, post-infectious hepatitis and mononucleosis, convalescent pneumonia—prenatal and postpartum fatigue—obesity—anemia

note: The use of SPARTASE is not intended to supplant specific treatment for organic disease or to substitute for specific indications for potassium.

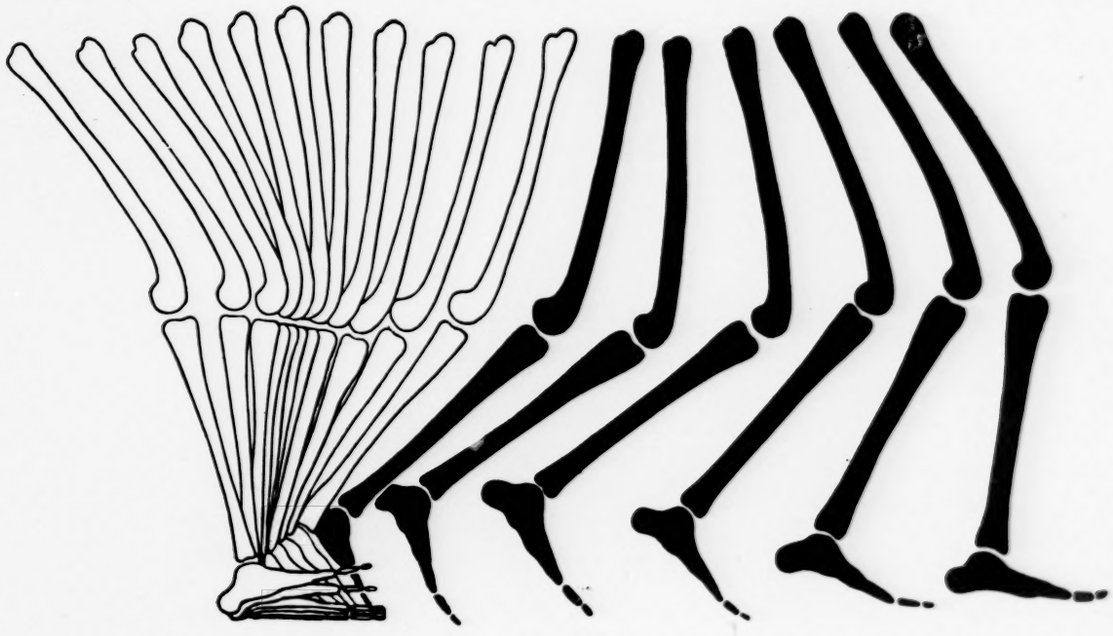
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references: 1. Kruse, C.A.: Northwest Med. *60*:597 (June) 1961. 2. Chesney, M.A., and Tullis, I.F.: Scientific Exhibit, Annual Meeting, American Medical Assoc., New York City, June 25-30, 1961. 3. Shaw, D.L., Jr.; Chesney, M.A.; Tullis, I.F., and Agersborg, H.P.K., Jr.: Paper read at Sixty-second Annual Meeting, American Therapeutic Society, New York City, June 22-25, 1961.

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Precautions

Depo-Medrol for local effect is contraindicated in the presence of acute infectious conditions. Infrequently, atrophic changes in the dermis may form shallow depressions in the skin at the injection site, but these usually disappear in a few months.

Depo-Medrol 40 mg. per cc.
Each cc. contains:

Medrol (methylprednisolone) acetate	40 mg.
Polyethylene glycol 4000	29 mg.
Sodium chloride	8.7 mg.
Myristyl-gamma-picolinium chloride	0.19 mg.
Water for injection	q.s.

Supplied: 1 cc. and 5 cc. vials

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Each cc. contains:

Medrol (methylprednisolone) acetate	20 mg.
Polyethylene glycol 4000	29.6 mg.
Sodium chloride	8.9 mg.
Myristyl-gamma-picolinium chloride	0.19 mg.
Water for injection	q.s.

Supplied: 5 cc. vials

1. Norcross, B. M., and Winter, J. A.: Methylprednisolone acetate: a single preparation suitable for both intra-articular and systemic use, New York J. Med. *61*:552 (Feb. 15) 1961.

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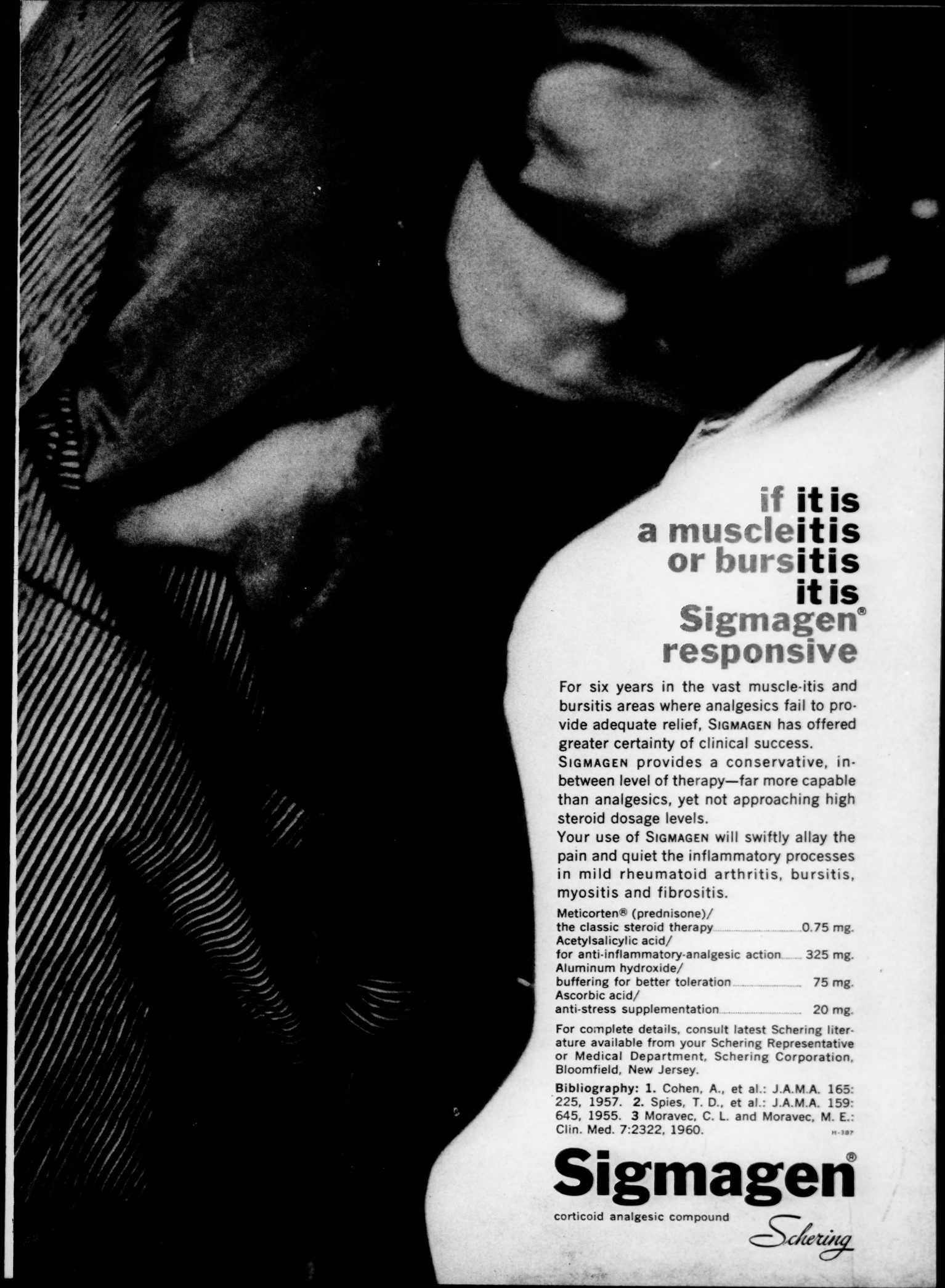
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Bibliography: 1. Cohen, A., et al.: J.A.M.A. 165: 225, 1957. **2.** Spies, T. D., et al.: J.A.M.A. 159: 645, 1955. **3** Moravec, C. L. and Moravec, M. E.: Clin. Med. 7:2322, 1960.

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Full thiazide therapy that goes easy on the potassium

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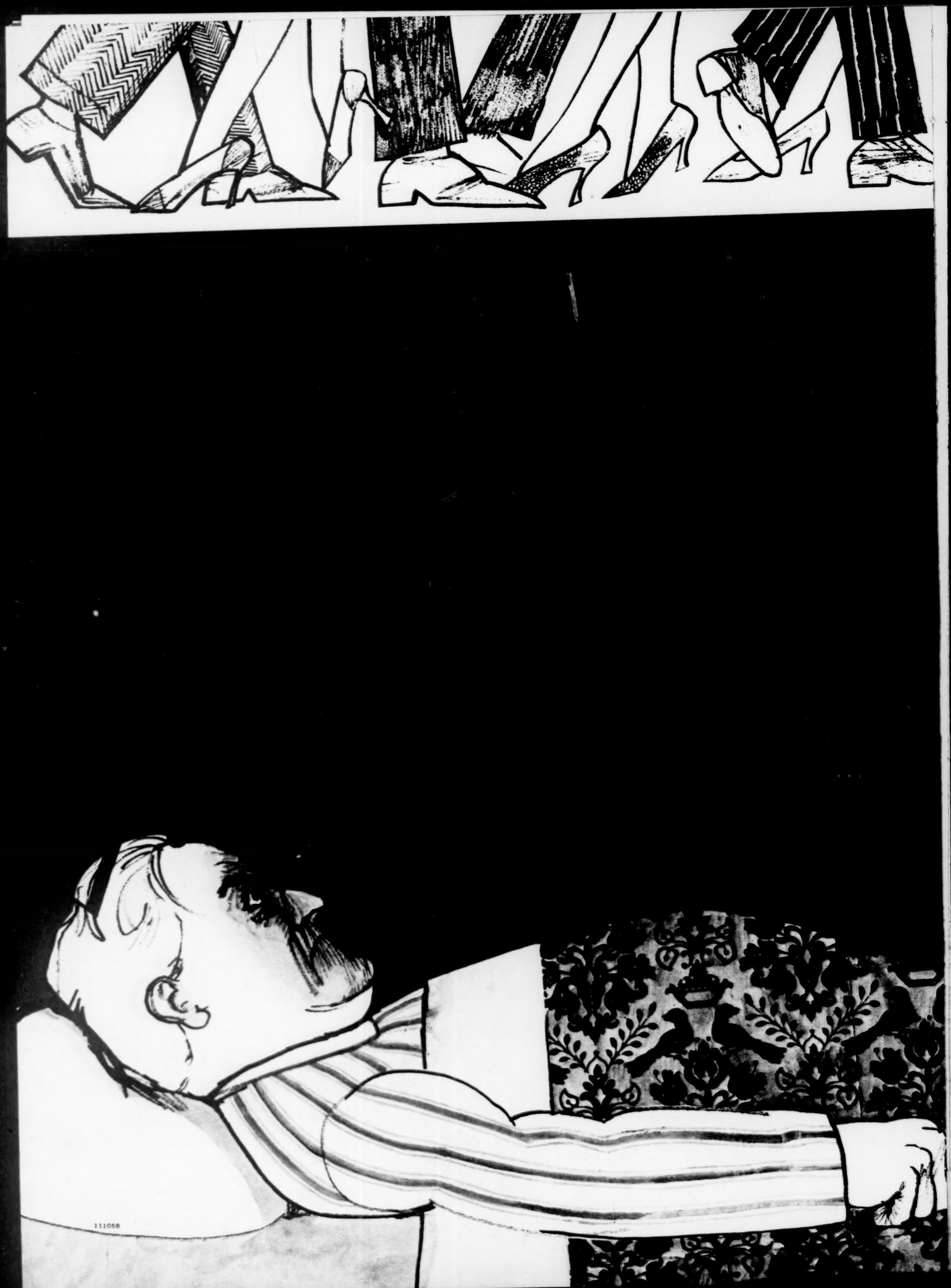
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Use Enduron with patients who have mild to moderate hypertension, or patients with edema (as in congestive heart failure, the nephrotic syndrome, hepatic cirrhosis, premenstrual tension, or steroid therapy). Observe its convenience and effectiveness. *We predict you'll be glad you used it.*

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1. ENDURON (*Methyclothiazide*) This long-acting diuretic produces enhanced sodium excretion per unit of potassium excreted and minimal potassium loss. It has amply demonstrated its merit as a primary measure in controlling mild ~~to~~ moderate hypertension.

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Together, these two components provide even greater antihypertensive action than with either alone. Blood pressure starts down steadily, and improvement should be substantial within 10 days. Use Enduronyl and see. Full literature available promptly on request: *contact Abbott Laboratories, North Chicago, Illinois.*

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Medrol... (methylprednisolone) a form for every use

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2 mg. in bottles of 30 and 100
4 mg. in bottles of 30, 100 and 500
16 mg. in bottles of 50

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40 mg. in 1 cc. Mix-O-Vial*

MEDROL MEDULES*
4 mg. in bottles of 30, 100 and 500 capsules
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DEPO-MEDROL*
acetate
40 mg./cc. in 1 cc. and 5 cc. vials
20 mg./cc. in 5 cc. vials



MEDROL
WITH ORTHOXINE*
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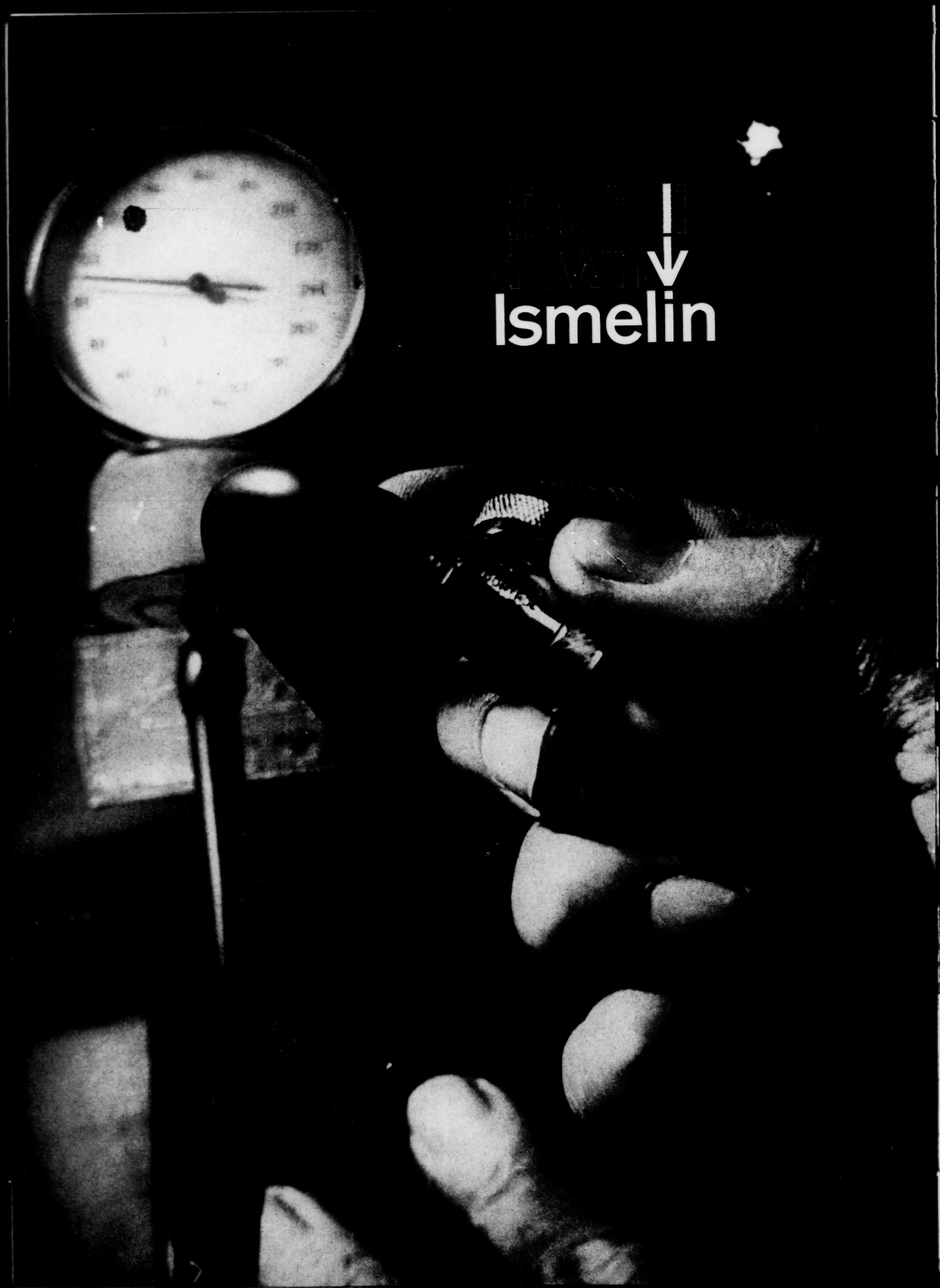
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MEDAPRIN* TABLETS
in bottles of 100 and 500

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Ismelin

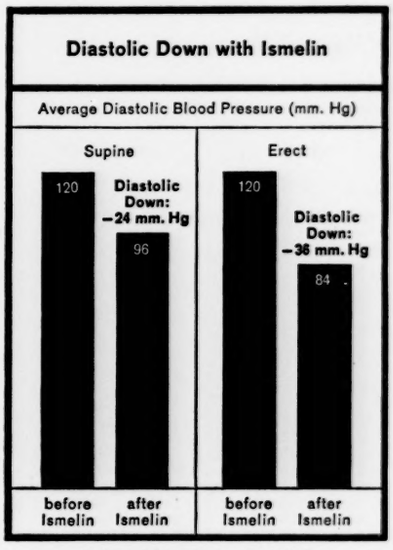
With Ismelin, "...a decrease in systolic and diastolic blood pressure in both supine and standing positions in all patients."[1]

Elevated diastolic pressure of "crucial importance."[2] Increased peripheral resistance, as reflected by elevation of diastolic blood pressure, has been described as: "The single most important factor in the production of the type of arterial hypertension with which the physician is usually concerned..."[2]

Ismelin lowers diastolic pressure after other treatments fail. Riven and Hall[1] studied Ismelin in 21 male hypertensive patients ranging in age from 30 to 69 years. Most patients were hospitalized initially, and most were treated with other antihypertensive drugs. (When therapy with Ismelin began, mecamylamine was discontinued in 7 patients receiving it.) Before treatment with Ismelin, all patients had diastolic pressures (supine and erect) of at least 100 mm. Hg "...despite other antihypertensive therapy including ganglionic blocking agents." Ismelin produced "...a decrease in systolic and diastolic pressure in both supine and standing positions in all [21] patients. In most cases there was a greater reduction in standing blood pressure. The average reduction in diastolic blood pressure was 24 mm. Hg supine and 36 mm. Hg standing. The development of tolerance was not observed."[1]

Advantages of Ismelin for your hypertensive patients

- Almost all forms of moderate to severe hypertension (including malignant hypertension and many cases of renal hypertension) can be managed with Ismelin — alone or in combination with other antihypertensive agents.
- Ismelin brings blood pressure down in many persons refractory to other antihypertensive agents.
- Ismelin lowers blood pressure in many patients who cannot be treated effectively with other potent agents because they cannot or will not tolerate the side effects.
- Patients need take Ismelin but once a day.



(Adapted from Riven and Hall[1])

- Most patients have been treated with Ismelin for prolonged periods without developing tolerance to it (although instances of tolerance have been reported).
- Smooth absorption of Ismelin results in predictable blood pressure responses.

For complete information about Ismelin (including dosage, cautions, and side effects), see current Physicians' Desk Reference or write CIBA, Summit, N. J.

Supplied: TABLETS, 10 mg. (pale yellow, scored) and 25 mg. (white, scored).

References: 1. Riven, S. S., and Hall, W.: South. M. J. 54:673 (June) 1961. **2.** Harrison, T. R., Adams, R. D., Bennett, I. L., Jr., Resnick, W. H., Thorn, G. W., and Wintrobe, M. M. (Editors): **Principles of Internal Medicine,** Vol. 2, Third Edition, The Blakiston Division, McGraw-Hill Book Company, Inc., New York, 1958, p. 1321. 2/2998 M9-1

ISMELIN® sulfate (guanethidine sulfate CIBA)

diastolic down **Ismelin®**

CIBA Summit, New Jersey



Put your low-back patient back on the payroll

Soma relieves stiffness —stops pain, too

YOUR CONCERN: Rapid relief from pain for your patient. Get him back to his normal activity, fast!

HOW SOMA HELPS: Soma provides direct pain relief while it relaxes muscle spasm.

YOUR RESULTS: With pain relieved, stiffness gone, your patient is soon restored to full activity—often in days instead of weeks.

Kestler reports in controlled study: *Average time for restoring patients to full activity: with Soma, 11.5 days; without Soma, 41 days.* (*J.A.M.A. Vol. 172, No. 18, April 30, 1960.*)

Soma is notably safe. Side effects are rare. Drowsiness may occur, but usually only in higher dosages. Soma is available in 350 mg. tablets. **USUAL DOSAGE: 1 TABLET Q.I.D.**

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SOMA®

(carisoprodol, Wallace)

Wallace Laboratories, Cranbury, New Jersey

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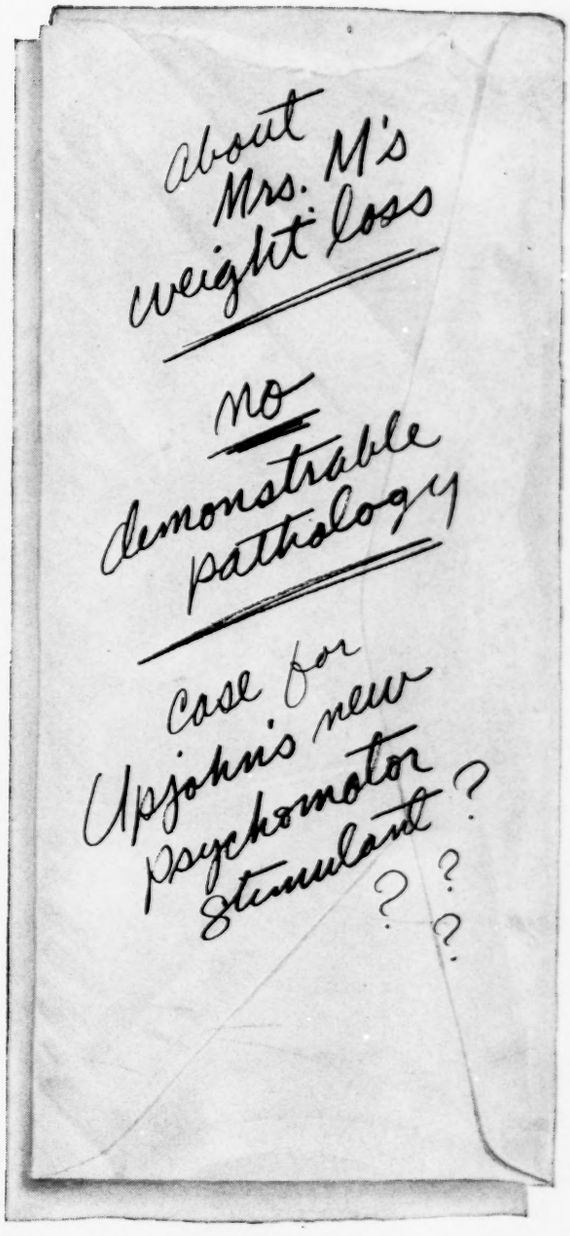
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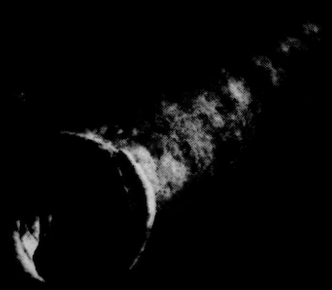
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*Trademark, Reg. U.S. Pat. Off. – brand of etryptamine acetate

SEE PAGE 40–41

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75th year



for potential ulcer...
to relieve tensions and to inhibit
hypermotility and hypersecretion

PATHIBAMATE®

PATHILON® (propantheline bromide Lederle) + the aminobamate

highly effective with minimal side effects for therapeutic/prophylactic [illegible] duodenal ulcer, gastric ulcer, gastrointestinal hypermotility, spastic and irritable colon, diarrhea, esophageal spasm, aerophagia, pylorospasm, gastrointestinal symptoms, gastric hypersecretion.

CONTRAINDICATIONS: glaucoma, pyloric obstruction, obstruction of the urinary bladder neck. Request complete information on indications, dosage, precautions and contraindications from your Lederle representative or write to Medical Advisory Department



for patent ulcer...
to relieve tensions and to inhibit
hypermotility and hypersecretion

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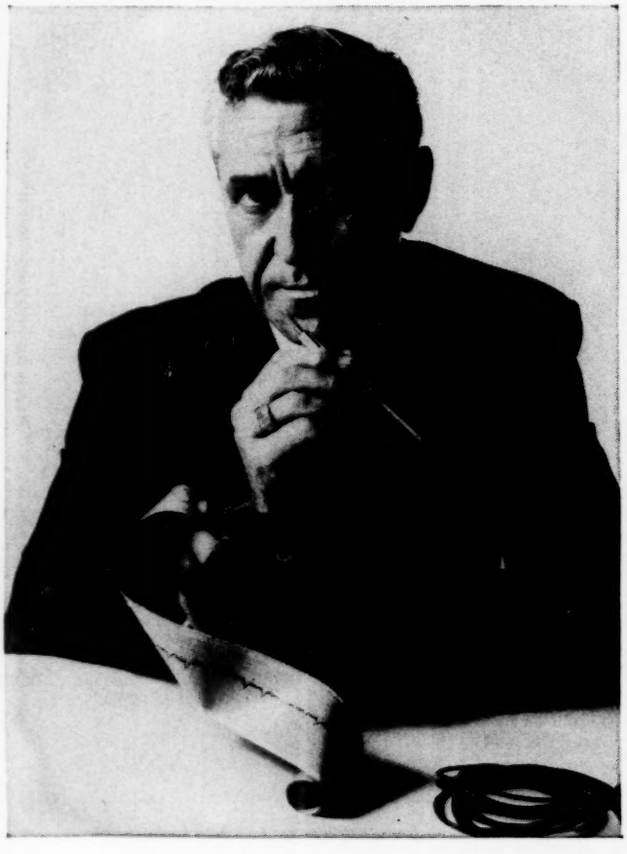
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Not objectively, as activity of the heart can be measured electrocardiographically.

The higher level of relief reported with this new corticosteroid is a subjective thing that must be seen, by you, in your own patients.

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See page 51 for description, indications, dosage, precautions, side effects, and how supplied.

The Upjohn Company, Kalamazoo, Michigan

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Kills pain.....stops tension

For neuralgias, dysmenorrhea, upper respiratory distress, and postsurgical conditions—new compound of Soma, phenacetin and caffeine kills pain, stops tension, reduces fever—gives more complete relief than other analgesics . . . acts fast, relief lasts four to six hours

Composition: 200 mg. Soma (carisoprodol), 160 mg. phenacetin, 32 mg. caffeine. **Dosage:** 1 or 2 tablets q.i.d. **Supplied:** Bottles of 50 apricot-colored, scored tablets.

Also Available As
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Soma Compound boosts the effectiveness of codeine. SOMA COMPOUND + CODEINE therefore contains only ¼ grain of codeine phosphate to relieve the more severe pain that usually requires ½ grain. Otherwise, its composition—and dosage—is the same as Soma Compound. Supplied in bottles of 50 white, lozenge-shaped tablets.

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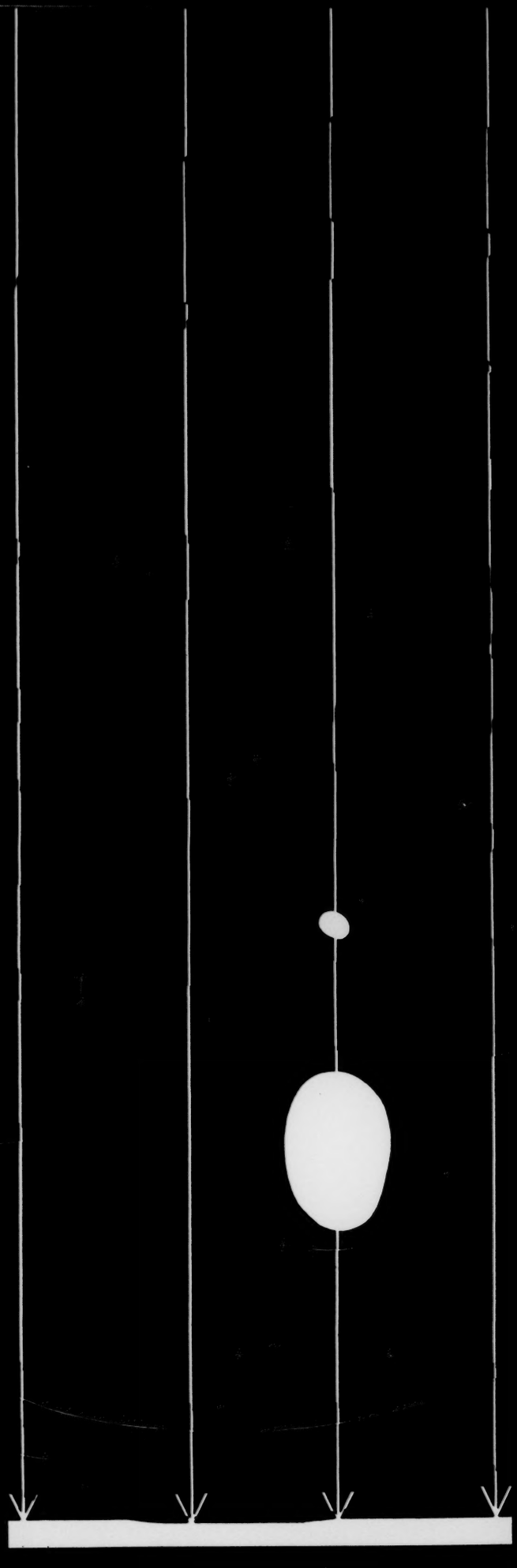


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excellent diagnostic accuracy (98.1 per cent in 1207 examinations[2])

administration convenient and economical

X-ray examination simple and rapid; repeat study rarely required

high safety margin; side effects few and invariably mild

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gallbladder function (concentrating ability) better demonstrated than with intravenous agents

fluoroscopy for spot films facilitated diagnosis of unusual abnormalities more frequent

How Supplied: Tablets of 500 mg., envelopes of 6 tablets, boxes of 5 and 25 envelopes; also bottles of 500.

1. Whitehouse, W. M.: Iopanoic acid, Ann. New York Acad. Sc. 78:809, July 2, 1959. 2. Baker, H. L., Jr., and Hodgson, J. R.: Further studies on the accuracy of oral cholecystography, Radiology 74:239, Feb. 1960.

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superior oral cholecystography and cholangiography

Telepaque (brand of iopanoic acid). trademark reg. U.S. Pat Off.

Winthrop LABORATORIES, NEW YORK 18, N.Y.

It is suggested that physicians consult Winthrop literature for information about dosage, precautions, side effects and contraindications.

FOUR HUNDRED MILLIGRAMS OF PURE

PAIN RELIEF

NEW analexin®-400

400 milligrams of phenyramidol HCl

THE ONLY SIGNIFICANT RESPONSE IS RELIEF FROM PAIN

EXCEEDINGLY EFFECTIVE "... The 85.1% incidence of effectiveness with the 400 mg. dose has exceeded the analgesic effectiveness of any other analgesic agent which we have studied to date, either alone or in combination.... The utilization of higher doses for short periods of time indicates that the medication has a large therapeutic range, and this is reflected in the high incidence of effectiveness and low likelihood of untoward reactions.

"The practicing physician translating this into his own needs may be completely confident of using a medication with an excellent predictability and a safe analgesic response."[1]

EXTRAORDINARY MARGIN OF SAFETY. *Analexin-400 is non-narcotic and not narcotic related;* thus, it presents no danger of habituation or any other reaction associated with the frequent use of narcotics. Nor will Analexin-400 produce sedation, mental confusion or depression occasionally observed with other analgesics or interneuronal blocking agents.[1-23]

INDICATIONS: Relief of pain in injury, low back pain, premenstrual cramping, dysmenorrhea, postoperative pain, and a wide variety of recurring and acute painful conditions.

DOSAGE: One capsule at onset of pain, followed by 1 capsule at intervals of 1 to 4 hours, as needed.

REFERENCES: From the Symposium, *Recent Concepts of Pain and Analgesia*, held in the Hall of States, American Hospital Association, Chicago, February 15, 1961: 1. Batterman, R. C.: *Non-Narcotic Analgesia in Ambulatory Patients.* 2. O'Dell, T. B.: *Experimental Parameters in the Evaluation of Analgesics.* 3. Miller, L. D.: *Distribution, Excretion and Metabolic Fate of Phenyramidol.* 4. Beisler, E.: *Preliminary Report of Experience with Phenyramidol for Dental Analgesia.* 5. Bader, G.: *Preliminary Report on the Use of Analexin for Dysmenorrhea in Telephone Operators.* 6. Taylor, S. L.: *Phenyramidol in General Hospital Orthopedics.* 7. Bodi, T.: *Pain Management Among Clinic Outpatients.* 8. Ramunis, J.: *Experience of an Industrial Surgeon with Phenyramidol.* 9. Kast, E. C.: *Methodological Considerations in the Clinical Evaluation of an Analgesic.* 10. Collopy, C. T.: *Preliminary Comparisons of Two Non-Narcotic Analgesic Agents in Hospitalized Orthopedic Patients.* 11. Cass, L. J.: *Report on the Analgesic and Calmative Effectiveness of Two Preparations on Patients with Acute and Chronic Pain.* 12. Lamphier, T. A.: *Intravenous Phenyramidol in the Management of Low Back Pain and Allied Disorders.* 13. O'Dell, T. B.: Chicago Med. *63*:9, 1961. 14. Kast, E. C.: Chicago Med. *63*:17, 1961. 15. Wainer, A. S.: J. Am. M. Women's A. *16*:218, 1961. 16. Batterman, R. C.: Ann. New York Acad. Sc. *86*:203, 1960. 17. O'Dell, T. B.: Ann. New York Acad. Sc. *86*:191, 1960. 18. O'Dell, T. B., *et al.*: J. Pharmacol. & Exper. Therap. *128*:65, 1960. 19. O'Dell, T. B., *et al.*: Fed. Proc. *18*:1694, 1959. 20. Gray, A. P., *et al.*: J. Am. Chem. Soc. *81*:4347, 1959. 21. Wainer, A. S.: Clin. Med. *7*:2331, 1960. 22. Clinical data in files of Medical Dept., Irwin, Neisler & Co., 1959. 23. Batterman, R. C., *et al.*: Am. J. Med. Sc. *238*:315, 1959.

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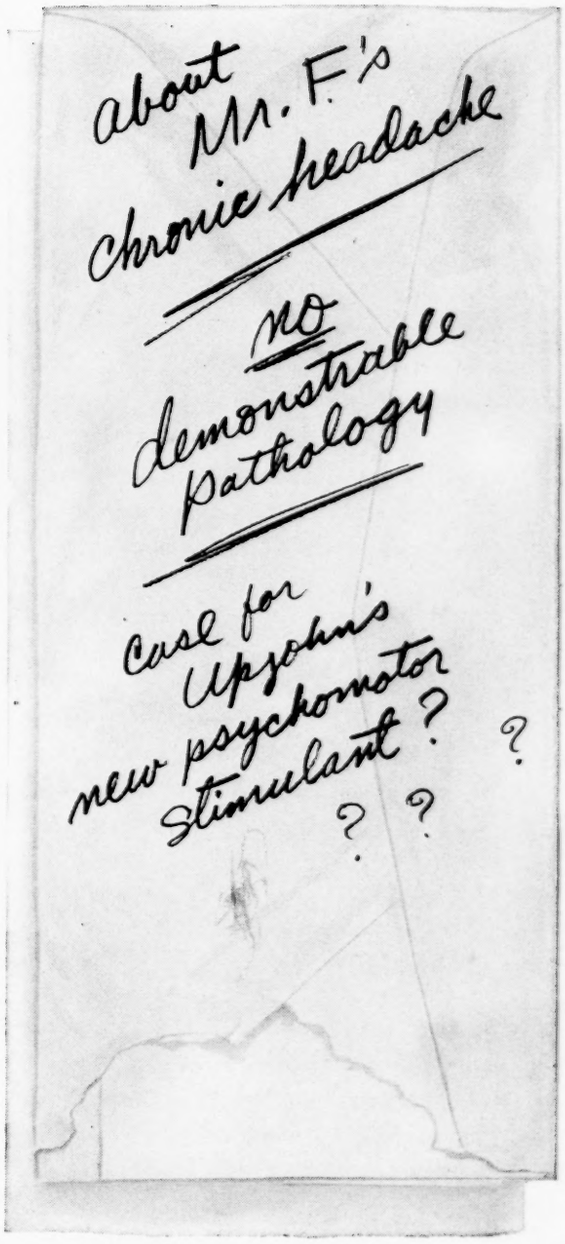
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Excerpts from a 2-year study of 87 patients

USE OF DEPROL IN THE OFFICE TREATMENT OF DEPRESSION*

"Eighty-seven patients were studied during a period of over 2 years. All were psychoneurotic, and all were seen in private psychiatric practice. Although diagnoses differed, the most prominent symptom in each case was severe depression... Patients ranged in age from 16 to 70 years; the greater portion were 20 to 40 years old.

"The usual starting dosage of Deprol† was 1 tablet 4 times a day... If necessary, this dosage was increased to 6 tablets per day, and then to 8."

Results

"All except 2 of the 87 patients treated with Deprol were definitely helped by this medication.

"Deprol was found most useful in patients with pronounced depressions characterized by apathy, withdrawal, and inability to perform. Such patients were relieved of their oppressive despondency and crying spells and became accessible to psychotherapy. They became more hopeful and more willing and able to expend effort to help themselves. They were able to sleep well, to enjoy their food again, to concentrate better, to make decisions and to return to normal activity...

"Unlike most other drugs used for depression, [Deprol] is also effective in controlling a wide spectrum of associated symptoms, particularly anxiety, tension, sleep disturbances, and psychosomatic complaints. Deprol does not depress appetite but permits its normal return as the patient improves. It is not a euphoriant; rather, patients taking the drug experience a return to a stable and normal mood."

Side Effects and Toxicity

During the two years of this study "...no side-effects were observed. Two patients who attempted suicide by ingesting, respectively, 40 and 30 tablets of Deprol experienced prolonged sleep with slight, transient fall in blood pressure, but they recovered without treatment and without sequelae."

Conclusion

"Deprol marks a definite step forward in the safe and effective treatment of depression."

*Ruchwarger, A.: M. Ann. District of Columbia 28:438, Aug. 1959.
†Supplied by WALLACE LABORATORIES, Cranbury, N. J.

▲Deprol▲®

Dosage: Usual starting dose is 1 tablet q.i.d. When necessary, this may be increased gradually up to 3 tablets q.i.d. With establishment of relief, the dose may be reduced gradually to maintenance levels.

Composition: 1 mg. 2-diethylaminoethyl benzilate hydrochloride (benactyzine HCl) and 400 mg. meprobamate.

Supplied: Bottles of 50 light-pink, scored tablets.

CD-5851

a pair of cardiac patients:



both are free of pain—but only one is on

DILAUDID®

(Dihydromorphinone HCl)

swift, sure analgesia normally unmarred by nausea and vomiting

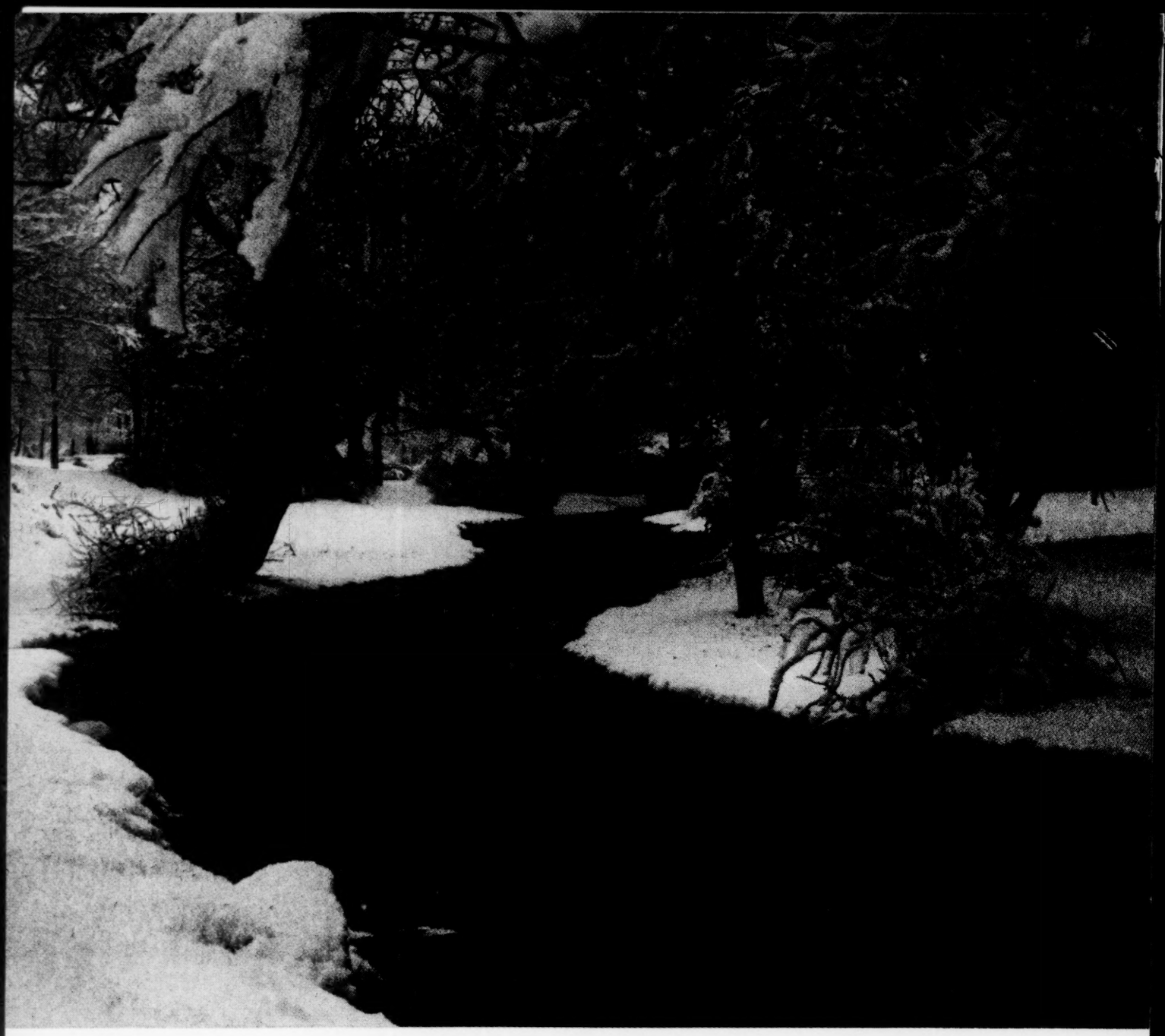
DILAUDID provides unexcelled analgesia in acute cardiovascular conditions. Onset of relief from pain is almost immediate. The high therapeutic ratio of DILAUDID is commonly reflected by lack of nausea and vomiting—and marked freedom from other side-effects such as dizziness and somnolence.

▲ by mouth ▲ by needle ▲ by rectum

2 mg., 3 mg., and 4 mg.

May be habit forming—usual precautions should be observed as with other opiate analgesics.

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Quietude for the Hypertensive

Gently—like the fall of snow—BUTISERPINE® lowers blood pressure and releases the patient from tension.

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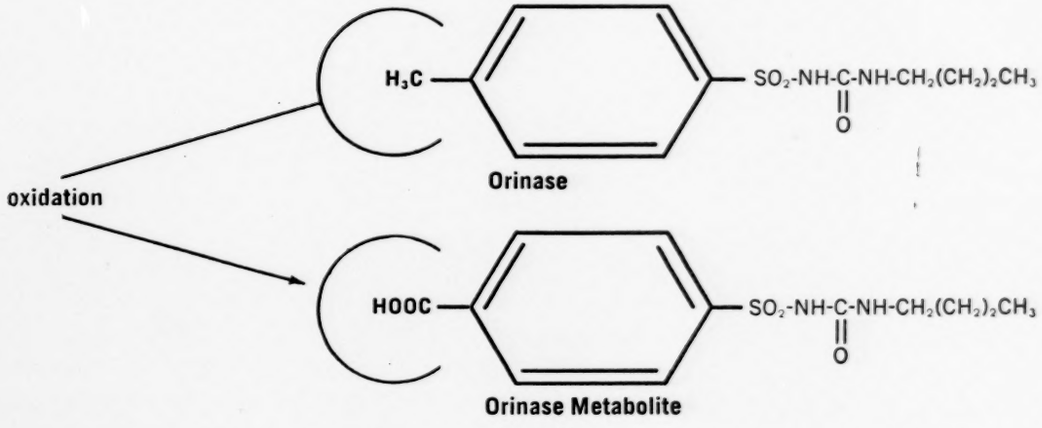
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Why is the methyl "governor" in Orinase so important?

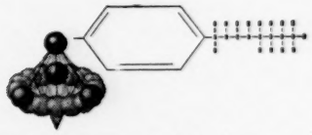
One of the most significant advantages of Orinase therapy is the rarity of associated hypoglycemic reactions.

This widely-reported clinical benefit is a function of the exclusive Orinase methyl "governor." Lending itself to ready oxidation (principally, it is thought, a hepatic process), the methyl group ensures prompt metabolic inactivation of the Orinase molecule. What actually happens is that a rapidly- and continuously-excreted carboxy-metabolite is produced that has no hypoglycemic activity at the existing levels.

As a result of the oxidation of its methyl group, Orinase shows a decline in activity soon after it reaches its effective peak in the plasma. Maintenance dosage serves to reduce blood sugar levels to normal, but rarely below that point, and there is no reported problem of accumulation.



Orinase*



An exclusive methyl "governor" minimizes hypoglycemia

Indications and effects: The clinical indication for Orinase is stable diabetes mellitus. Its use brings about the lowering of blood sugar; glycosuria diminishes, and such symptoms as pruritus, polyuria, and polyphagia disappear.

Dosage: There is no fixed regimen for initiating Orinase therapy. A simple and effective method is as follows: *First day*—6 tablets; *second day*—4 tablets; *third day*—2 tablets. The daily dose is then adjusted—raised, lowered or maintained at the two-tablet level, whichever is necessary to maintain optimum control.

Patients receiving insulin (less than 20 units)—discontinue insulin and institute Orinase; (20 to 40 units)—initiate Orinase with a concurrent 30 to 50% reduction in insulin dose with a further careful reduction as response to Orinase is observed; (more than 40 units)—reduce insulin by 20% and initiate Orinase with a further careful reduction in insulin dosage as response to Orinase is observed. In candidates for combined Orinase-insulin therapy, an individualized schedule is usually obtainable during a trial course of two or more weeks.

Contraindications and side effects: Orinase is contraindicated in patients having juvenile or growth-onset, unstable or brittle types of diabetes mellitus; history of diabetic coma, fever, severe trauma or gangrene.

Side effects are mild, transient and limited to approximately 3% of patients. Hypoglycemia and toxic reactions are extremely rare. Hypoglycemia is most likely to occur during the period of transition from insulin to Orinase. Other untoward reactions to Orinase are usually not of a serious nature and consist principally of gastrointestinal disturbances, headache, and variable allergic skin manifestations. The gastrointestinal disturbances (nausea, epigastric fullness, heartburn) and headache appear to be related to the size of the dose, and they frequently disappear when dosage is reduced to maintenance levels or the total daily dose is administered in divided portions after meals. The allergic skin manifestations (pruritus, erythema, and urticarial, morbilliform, or maculopapular eruptions) are transient reactions, which frequently disappear with continued drug administration. However, if the skin reactions persist, Orinase should be discontinued.

Clinical toxicity: Orinase appears to be remarkably free from gross clinical toxicity on the basis of experience accumulated during more than four years of clinical use. Crystalluria or other untoward effects on renal function have not been observed. Long-term studies of hepatic function in humans and experience in over 650,000 diabetics have shown Orinase to be remarkably free of hepatic toxicity. There has been reported only one case of cholestatic jaundice related to Orinase administration, which occurred in a patient with pre-existing liver disease and which rapidly reversed upon discontinuance of the drug.

Each tablet contains:
Tolbutamide ..... 0.5 Gm.

Supplied: In bottles of 50.

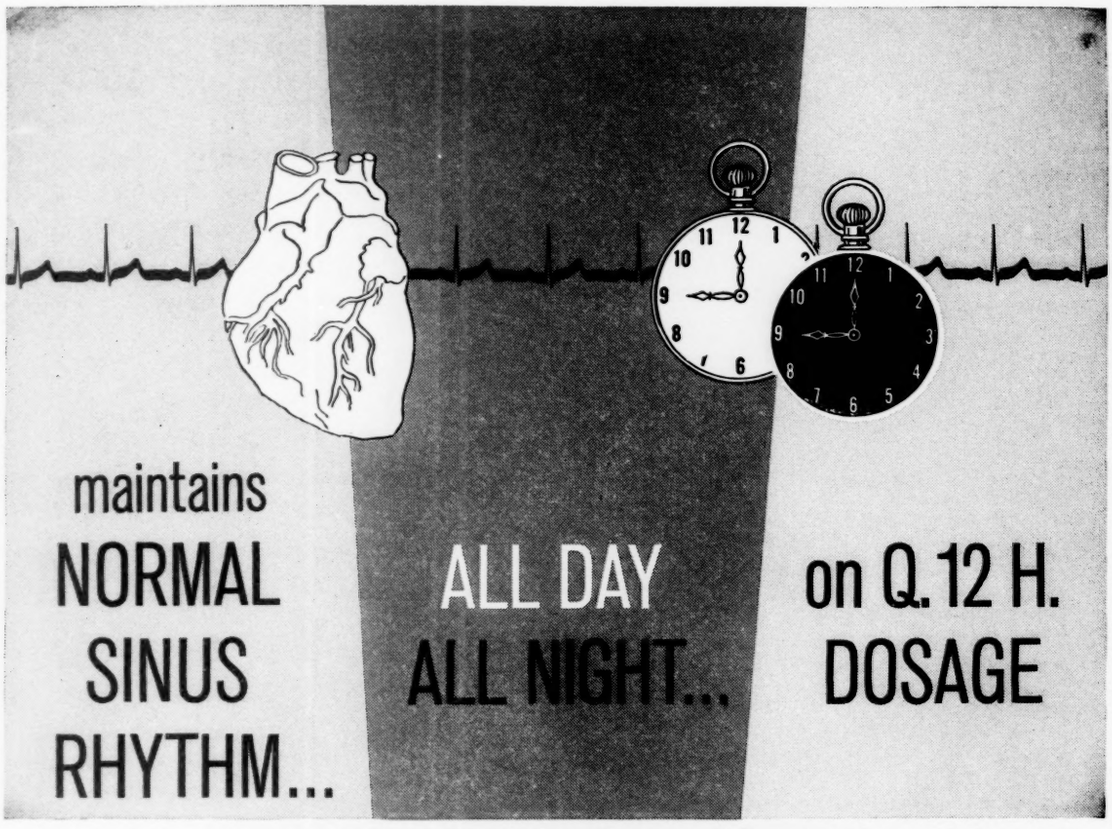
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June, 1961

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QUINAGLUTE®

DURA-TABS®

exclusive oral Sustained Medication*
Quinidine Gluconate 5 gr. (0.33 Gm.)

IN CARDIAC ARRHYTHMIAS[1-10]

Maximum efficacy: maintains effective quinidine blood levels all day, all night. **Better tolerated:** because quinidine gluconate is 10 times as soluble as the sulfate, and only part of daily Dura-Tab dosage contacts gastric mucosa. **Maximum convenience:** given q. 12 h.—no night dosage needed.

DOSAGE: for conversion of auricular fibrillation to normal sinus rhythm, in most cases, 2 Dura-Tabs 3 or 4 times a day, for 2 to 3 days; longer periods are required in some patients. **For maintenance,** 1 or 2 Dura-Tabs q. 12 h. in most patients . . . Bottles of 30, 100 and 250 Quinaglute Dura-Tabs.

For SAMPLES and complete literature[1-10] giving indications, cautions, etc., write

REFER TO PDR Page 821

WYNN PHARMACAL CORPORATION
Lancaster Ave. at 51st St., Philadelphia, Pa.

*U.S. Patent 2,895,881

also available **INJECTABLE QUINAGLUTE**

for superior control of infection and pain...

UROBIOTIC assures effective antimicrobial levels in **tissue, urine, and blood stream**... because only UROBIOTIC provides the broad-spectrum action of OXYTETRACYCLINE, notable for its relatively high urinary concentration, excellent toleration, and proven effectiveness against even so troublesome an invader as Pseudomonas; SULFAMETHIZOLE — an unusually soluble, highly active sulfonamide; PHENAZOPYRIDINE — for effective local analgesia.

UROBIOTIC®

TERRAMYCIN®—SULFONAMIDE—ANALGESIC

permeates the urinary tract



IN BRIEF

INGREDIENTS: Each Urobiotic capsule contains 125 mg. Terramycin® (oxytetracycline), as the hydrochloride, with 125 mg. glucosamine HCl; 250 mg. sulfamethizole; and 50 mg. phenazopyridine HCl.

INDICATIONS: Genitourinary infections caused by susceptible organisms; and prophylactically, before and after genitourinary or pelvic surgery, following instrumentation procedures, during the use of retention catheters, and in patients with conditions such as cord bladder or cystocele.

DOSAGE: In adults, a dose of 1 or 2 capsules four times daily, depending upon the severity and response of the infection. In children 60 to 100 lbs., 1 capsule four times daily; in children under 60 lbs., 1 capsule three times daily. Therapy should be continued for a minimum of 7 days or until bacteriologic cure is effected in acute urinary tract infections.

CONTRAINDICATIONS: Urobiotic may be contraindicated in patients with chronic glomerulonephritis, hepatitis, hepatic failure, uremia, and obstructive lesions of the urinary tract, and should not be used in patients sensitive to any of its components.

PRECAUTIONS: The use of broad-spectrum antibiotics may, in rare cases, result in an overgrowth of nonsusceptible organisms, such as monilia or staphylococci. Should such superinfection occur, therapy with Urobiotic should be discontinued and specific therapy instituted as shown by susceptibility testing. The use of sulfonamides may cause renal crystalluria as well as other toxic or sensitivity reactions. If any of these occur, discontinue use.

SUPPLIED: Urobiotic capsules, yellow-and-grey, bottles of 50.

More detailed professional information available on request.

Science for the world's well-being® Pfizer PFIZER LABORATORIES Division, Chas. Pfizer & Co., Inc. New York 17, N. Y.

STOPS THE ASTHMA ATTACK IN MINUTES...FOR HOURS... ORALLY

ELIXOPHYLLIN®

RAPID RELIEF IN MINUTES —in 15 minutes[1,2,3] mean theophylline blood levels are comparable to I. V. aminophylline—so that severe attacks have been terminated in 10 to 30 minutes.[1,4,5,6] **Note:** *With Elixophyllin the patient can learn to abort an attack in its incipient stage.*

INHERENT SUSTAINED ACTION —After absorption theophylline is slowly eliminated during a 9-hour period.[7] Clinically *proved* relief and protection day and night with t.i.d. dosage.[1,3-6,8,9]

NO UNNEEDED SIDE EFFECTS —Since Elixophyllin does not need "auxiliaries," it contains no ephedrine—no barbiturate—no iodide—no steroid. *Gastric distress is rarely encountered.*[8,9]



Each tablespoonful (15 cc.) contains theophylline 80 mg. (equivalent to 100 mg. aminophylline) in a hydro-alcoholic vehicle (alcohol 20%).

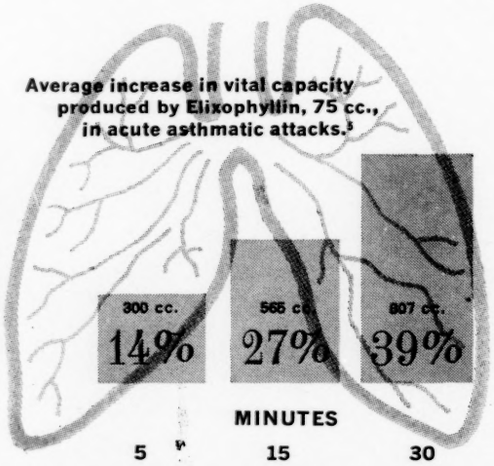
ACUTE ATTACKS:

single dose of 75 cc. for adults; 0.5 cc. per lb. of body weight for children.

24 HOUR CONTROL:

for adults 45 cc. doses before breakfast, at 3 P.M., and before retiring; after two days, 30 cc. doses. Children, first 6 doses 0.3 cc.—then 0.2 cc. per lb. of body weight as above.

Average increase in vital capacity produced by Elixophyllin, 75 cc., in acute asthmatic attacks.[5]



REFERENCES: 1. Kessler, F.: Connecticut M.J. *21*:205 (March) 1957. 2. Schluger, J.; McGinn, J.T., and Hennessy, D.J.: Am. J. Med. Sci. *233*:296 (March) 1957. 3. Kessler, F.: Med. Times (Oct.) 1959. 4. Burbank, B.; Schluger, J., and McGinn, J.: Am. J. Med. Sci. *234*:28 (July) 1957. 5. Spielman, A.D.: Ann. Allergy *15*:270 (June) 1957. 6. Greenbaum, J.: Ann. Allergy (May-June) 1958. 7. Waxler, S.H., and Shack, J.A.: J.A.M.A. *143*:736 (1950). 8. Bickerman, H.A., and Barach, A.L., in Modell, W.: Drugs of Choice 1960-1961, St. Louis, The C.V. Mosby Company, 1960, p. 516. 9. Wilhelm, R.E., Conn, H.F.: in Current Therapy—1961, Philadelphia, W.B. Saunders Company, p. 417.

Patent Pending

Reprints on request





when urinary tract infections present a therapeutic challenge...

CHLOROMYCETIN®

(chloramphenicol, Parke-Davis)

Often recurrent...often resistant to treatment, urinary tract infections are among the most frequent and troublesome types of infections seen in clinical practice.[1,2] In such infections, successful therapy is usually dependent on identification and susceptibility testing of invading organisms, administration of appropriate antibacterial agents, and correction of obstruction or other underlying pathology.

Of these agents, one author reports: "Chloramphenicol still has the widest and most effective activity range against infections of the urinary tract. It is particularly useful against the coliform group, certain Proteus species, the micrococci and the enterococci."[1] CHLOROMYCETIN is of particular value in the management of urinary tract infections caused by *Escherichia coli* and *Aerobacter aerogenes*.[3] In addition to these clinical findings, the wide antibacterial range of CHLOROMYCETIN continues to be confirmed by recent *in vitro* studies.[4-6]

CHLOROMYCETIN (chloramphenicol, Parke-Davis) is available in various forms, including Kapseals® of 250 mg., in bottles of 16 and 100. See package insert for details of administration and dosage.

Warning: Serious and even fatal blood dyscrasias (aplastic anemia, hypoplastic anemia, thrombocytopenia, granulocytopenia) are known to occur after the administration of chloramphenicol. Blood dyscrasias have occurred after both short-term and prolonged therapy with this drug. Bearing in mind the possibility that such reactions may occur, chloramphenicol should be used only for serious infections caused by organisms which are susceptible to its antibacterial effects. Chloramphenicol should not be used when other less potentially dangerous agents will be effective, or in the treatment of trivial infections, such as colds, influenza, or viral infections of the throat, or as a prophylactic agent. *Precautions:* It is essential that adequate blood studies be made during treatment with the drug. While blood studies may detect early peripheral blood changes, such as leukopenia or granulocytopenia, before they become irreversible, such studies cannot be relied upon to detect bone marrow depression prior to development of aplastic anemia.

References: (1) Malone, F. J., Jr.: *Mil. Med.* **125**:836, 1960. (2) Martin, W. J.; Nichols, D. R., & Cook, E. N.: *Proc. Staff Meet. Mayo Clin.* **34**:187, 1959. (3) Ullman, A.: *Delaware M. J.* **32**:97, 1960. (4) Petersdorf, R. G.; Hook, E. W.; Curtin, J. A., & Grossberg, S. E.: *Bull. Johns Hopkins Hosp.* **108**:48, 1961. (5) Jolliff, C. R.; Engelhard, W. E.; Ohlsen, J. R.; Heidrick, P. J., & Cain, J. A.: *Antibiotics & Chemother.* **10**: 694, 1960. (6) Lind, H. E.: *Am. J. Proctol.* **11**:392, 1960.

77361

PARKE-DAVIS

PARKE, DAVIS & COMPANY, Detroit 32, Michigan

If you were treating 25 pregnant, habitual aborters right now, what rate of success could you anticipate?

Lipp[1] administered DEPO-PROVERA to 25 pregnant, habitual aborters who totalled 94 previous successive abortions. Each patient had been under medical treatment for 8 to 48 months. DEPO-PROVERA was started between the third and eighth weeks of pregnancy. Six patients received an average of 5.7 injections of 25 mg. through the twentieth week, and 19 received an average of 4.4 injections of 50 mg. during the same period of time.

The results: The first group of 6 patients delivered 4 viable infants (67% salvage) while the second group of 19 patients delivered 16 (84% salvage). No side effects occurred and there was no evidence of female fetal masculinization.

Consider Intramuscular DEPO-PROVERA and Oral PROVERA for habitual aborters in your own practice: □ *in parenteral form—a single 50 mg. injection produces a progestational effect for up to 16 days.* □ *in oral form —effective action with small oral dose.* □ *no significant side effects reported during extensive clinical study.*

1. Lipp, R.G.: Habitual Abortion—Treatment with Parenteral Medroxyprogesterone Acetate, to be published.

Upjohn
75th year

Brief Basic Information

	Description	Indications	Dosage: Threatened abortion	Dosage: Habitual abortion	Supplied	Precautions
Oral Provera*	Upjohn brand of medroxyprogesterone acetate.	Threatened and habitual abortion, infertility, secondary amenorrhea, functional uterine bleeding.	10 to 30 mg. daily until acute symptoms subside.	1st trim. 10 mg. daily 2nd trim. 20 mg. daily 3rd trim. 40 mg. daily, through 8th month.	2.5 mg. scored, pink tablets, bottles of 25; 10 mg scored white tablets, bottles of 25 and 100.	Clinically, Provera is well tolerated. No significant untoward effects have been reported. Animal studies show that Provera possesses adrenocorticoid-like activity. While such adrenocorticoid action has not been observed in human subjects, patients receiving large doses of Provera continuously for prolonged periods should be observed closely. Likewise, large doses of Provera have been found to produce some instances of female fetal masculinization in animals. Although this has not occurred in human beings, the possibility of such an effect, particularly with large doses over a long period of time, should be considered. Provera, administered alone or in combination with estrogens, should not be employed in patients with abnormal uterine bleeding until a definite diagnosis has been established and the possibility of genital malignancy has been eliminated.
I. M. Depo-Provera*	Each cc. contains: Medroxyprogesterone acetate, 50 mg., Polyethylene glycol 4000, 28.8 mg., Polysorbate 80, 1.92 mg.; Sodium chloride, 8.65 mg., Methylparaben, 1.73 mg., Propylparaben, 0.19 mg., Water for injection, q.s.	Threatened and habitual abortion.	50 mg. I.M. daily while symptoms are present, followed by 50 mg. weekly through 1st trimester or until fetal viability is evident.	1st trim. 50 mg. I.M. weekly 2nd trim. 100 mg. I.M. q. 2 wks. 3rd trim. 100 mg. I.M. q. 2 wks. through 8th month.	Sterile aqueous suspension for intramuscular use only, 50 mg. per cc., in 1 cc. and 5 cc. vials.	

*Trademark, Reg. U.S. Pat. Off.

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80%
fetal salvage
in 25
habitual
aborters
with
94 previous,
successive
abortions[1]

Intramuscular Depo-Provera

Oral Provera



because
DIABETES IS FOR LIFE
start with Diabinese®
BRAND OF CHLORPROPAMIDE

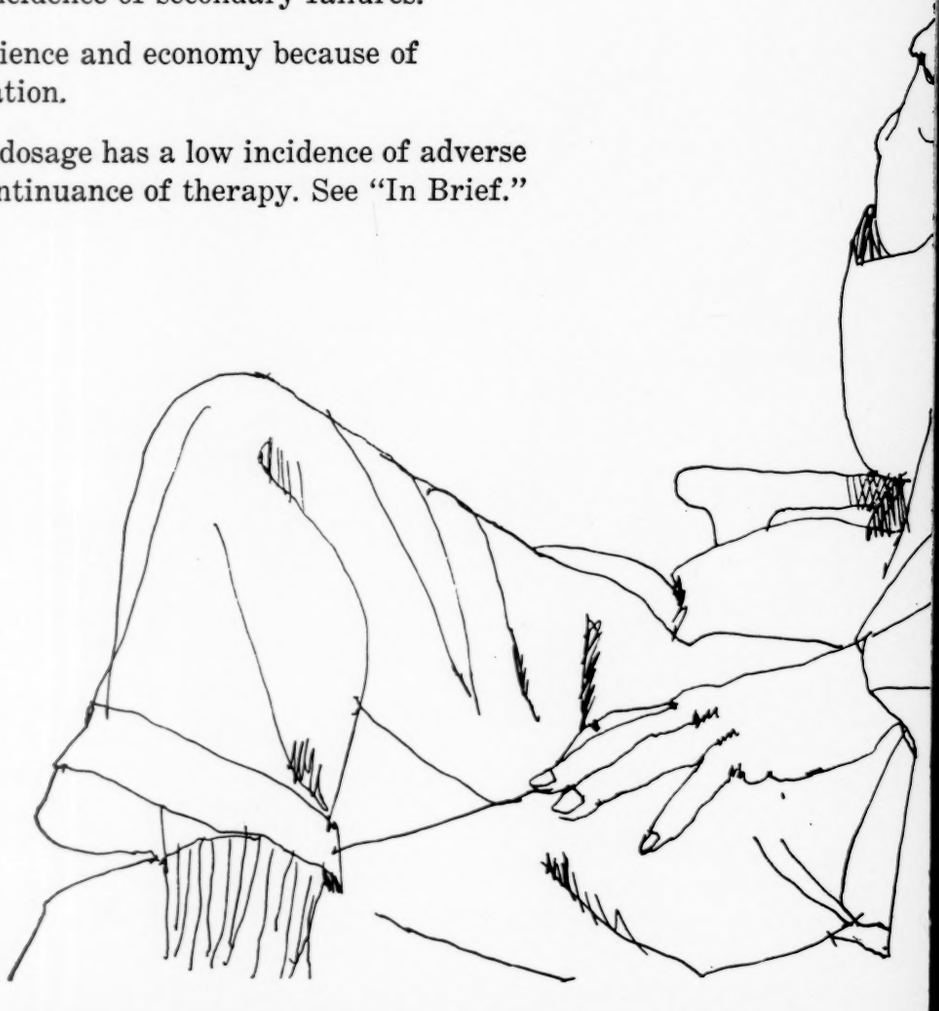
for maximum assurance
of continuing success
with oral therapy

long-term use continues to demonstrate that DIABINESE

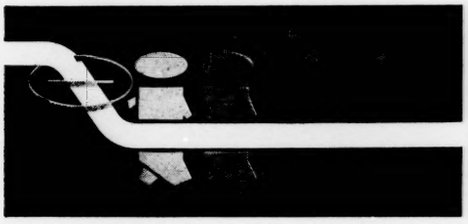
has a comparatively low incidence of secondary failures.

provides maximum convenience and economy because of *once-a-day* oral administration.

at presently recommended dosage has a low incidence of adverse effects which require discontinuance of therapy. See "In Brief."



when more than "diet alone" is needed by the maturity-onset diabetic



start with

Diabinese®

BRAND OF CHLORPROPAMIDE

the oral antidiabetic most likely to succeed

economical once-a-day dosage



IN BRIEF

DIABINESE, a potent sulfonylurea, provides smooth, long-lasting control of blood sugar permitting economy and simplicity of low, once-a-day dosage. Moreover, DIABINESE often works where other agents have failed to give satisfactory control.

INDICATIONS: Uncomplicated diabetes mellitus of stable, mild or moderately severe nonketotic, maturity-onset type. Certain "brittle" patients may be helped to smoother control with reduced insulin requirements.

ADMINISTRATION AND DOSAGE: Familiarity with criteria for patient selection, continued close medical supervision, and observance by the patient of good dietary and hygienic habits are essential.

As with insulin, DIABINESE dosage must be regulated to individual patient requirements. Average maintenance dosage is 100-500 mg. daily. For most patients the recommended starting dose is 250 mg. given once daily. Geriatric patients should be started on 100-125 mg. daily. A priming dose is not necessary and should not be used; most patients should be maintained on 500 mg. or less daily. Maintenance dosage above 750 mg. should be avoided. Before initiating therapy, consult complete dosage information.

SIDE EFFECTS: In the main, side effects, e.g., hypoglycemia, gastrointestinal intolerance, and neurologic reactions, are related to dosage. They are not encountered frequently on presently recommended low dosage. There have been, however, occasional cases of jaundice and skin eruptions primarily due to drug sensitivity; other side effects which may be idiosyncratic are occasional diarrhea (sometimes sanguineous) and hematologic reactions. Since sensitivity reactions usually occur within the first six weeks of therapy, a time when the patient is under very close supervision, they may be readily detected. Should sensitivity reactions be detected, DIABINESE should be discontinued.

PRECAUTIONS AND CONTRAINDICATIONS: If hypoglycemia is encountered, the patient must be observed and treated continuously as necessary, usually 3-5 days, since DIABINESE is not significantly metabolized and is excreted slowly. DIABINESE as the sole agent is not indicated in juvenile diabetes mellitus and unstable or severely "brittle" diabetes mellitus of the adult type. Contraindicated in patients with hepatic dysfunction and in diabetes complicated by ketosis, acidosis, diabetic coma, fever, severe trauma, gangrene, Raynaud's disease, or severe impairment of renal or thyroid function.

DIABINESE may prolong the activity of barbiturates. An effect like that of disulfiram has been noted when patients on DIABINESE drink alcoholic beverages.

SUPPLIED: As 100 mg. and 250 mg. scored chlorpropamide tablets.

More detailed professional information available on request.

Science for the world's well-being®

Pfizer

PFIZER LABORATORIES
Division, Chas. Pfizer & Co., Inc.
New York 17, New York



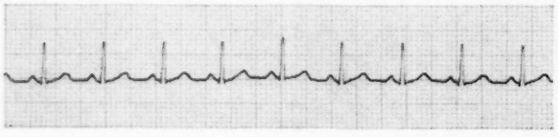
"makes the rounds" with you

In and out of elevators . . . up ramps and down corridors . . . from one room to another . . . the Sanborn "100M Viso-Cardiette®" goes wherever you need it. Its mobility is matched by its versatility in providing two speeds (25 or 50 mm/sec.), three recording sensitivities, and provision for recording and monitoring other phenomena. Cabinet is handsome mahogany or durable plastic laminate.

For office or laboratory use, the "100M Viso" provides the same instrument in a desk-top mahogany case. And for house calls, the Sanborn "300 Visette®" weighs only 18 pounds complete and can be easily carried by anyone.

No-obligation 15-day trial plan and convenient time payment may be arranged. Contact your nearest Sanborn Branch Office or Service Agency, or write Manager, Clinical Instrument Sales, at the main office.

Sanborn service lasts long after the sale . . . from people who know your ECG and value your satisfaction.



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Hostile Environment...



The fungus, protozoa and bacteria that commonly cause mild and severe leukorrhea require a vaginal pH of 5 to 12 for proliferation.

Trimagill creates a hostile environment! It produces a pH of 2.0 to 2.5—*the three principal infecting organisms cannot live in this acid range.*

Trimagill is well tolerated and has been proved effective in thousands of cases of leukorrhea, vaginitis, cervicitis, moniliasis and mixed infections. No untoward reactions that would require discontinuation of treatment were reported. At times denuded mucous membranes are so irritated that Trimagill may give a *temporary* burning sensation. This is usually short lived.

Trimagill does not foster resistant mutants or result in monilia overgrowth. Trimagill may be used during menstruation.

CONTENTS: Tartaric Acid, Citric Acid, Boric Acid, Dextrose, Potassium Alum, Potassium Bitartrate and Adhesives.

SUPPLIED: Powder: 5-oz. Plastic Insufflator Bottles; Vaginal Inserts: Boxes of 24.

NOTE: Consult package circular for information on dosage and instructions for use.

Write for descriptive literature.

TRIMAGILL®*

POWDER • VAGINAL INSERTS

THE S. E. MASSENGILL COMPANY

Bristol, Tennessee • New York • Kansas City • San Francisco

*Patent applied for.



Protects the angina patient better than vasodilators alone

Unless the coronary patient's ever-present anxiety about his condition can be controlled, it can easily induce an anginal attack or, in cases of myocardial infarction, can delay recovery.

This is why Miltrate gives better protection for the heart than vasodilators alone in coronary insufficiency, angina pectoris and postmyocardial infarction.

Miltrate contains PETN (pentaerythritol tetranitrate), acknowledged as basic therapy for long-acting vasodilation. . . .

What is more important—Miltrate provides Miltown, a tranquilizer which, unlike phenobarbital, relieves tension in the apprehensive angina patient without inducing daytime fogginess.

Thus, your patient's cardiac reserve is protected against his fear and concern about his condition; his operative arteries are dilated to enhance myocardial blood supply—and he can carry on normal activities more effectively since his mental acuity is unimpaired by barbiturates.

REFERENCES: **1.** Ellis, L. B. *et al.:* Circulation *17*:945, May 1958. **2.** Friedlander, H. S.: Am. J. Cardiol. *1*:395, Mar. 1958. **3.** Riseman, J.E.F.: New England J. Med. *261*:1017, Nov. 12, 1959. **4.** Russek, H. I. *et al.:* Circulation *12*:169, Aug. 1955. **5.** Russek, H. I.: Am. J. Cardiol. *3*:547, April 1959. **6.** Tortora, A. R.: Delaware M. J. *30*:298, Oct. 1958. **7.** Waldman, S. and Pelner, L.: Am. Pract. & Digest Treat. *8*:1075, July 1957.

Supplied: Bottles of 50 tablets. Each tablet contains 200 mg. Miltown and 10 mg. pentaerythritol tetranitrate.

Dosage: 1 or 2 tablets q.i.d. *before meals* and at bedtime, according to individual requirements.

CML-3619

Miltrate®

Miltown® (meprobamate) + PETN

WALLACE LABORATORIES / *Cranbury, N. J.*

FOR COUGH AND COLD DEMONS...



NON-NARCOTIC

ULO®

for control of acute cough regardless of etiology

ULOMINIC®

for control of acute cough and associated allergic reactions

ULOGESIC®

for control of acute cough and for relief from associated muscular aches, pain and fever

ULO

INHIBITS COUGH IMPULSE FOR 4 TO 8 HOURS
the threshold of the medullary cough center is elevated while the cough reflex is not abolished.

ULOMINIC

COUNTERACTS IRRITATION IN PHARYNX, LARYNX, TRACHEA AND BRONCHI
inhibits tendency of histamine to cause edema of the nasopharyngeal mucosa, local irritation, and vasodilation.

RELIEVES CONGESTION
reduces postnasal discharge, lessens irritation to pharyngeal and laryngeal membranes.

ULOGESIC

MAKES VOLUNTARY COUGH MORE PRODUCTIVE
loosens and liquefies mucus, soothes irritated bronchial mucosa.

Ulogesic enlarges the therapeutic dimensions of Ulominic

ALLEVIATES ASSOCIATED ACHES AND DISCOMFORTS AND ABORTS FEVER
elevates the pain threshold with an analgesic potency the same as acetanilid, with much less toxicity.

ULO®
non-narcotic antitussive molecule chlophedianol HCl

DIAFEN®
fast-acting antihistaminic diphenylpyraline HCl

PHENYLEPHRINE HCl
sympathomimetic

GLYCERYL GUAIACOLATE
expectorant and demulcent

APAP
acetyl-p-aminophenol analgesic and antipyretic

FORMULAS:

ULO SYRUP—Each 5 ml. teaspoonful contains:

chlophedianol • HCl* [alpha-(2-dimethylaminoethyl)-o-chlorobenzhydrol • HCl]	25 mg.
chloroform, U.S.P.	0.001 ml.

Alcohol 6.65 per cent in a pleasant flavored syrup base

ULOMINIC® SYRUP — Each teaspoonful (5 cc) contains:

chlophedianol HCl* [alpha-(2-dimethylaminoethyl)-o-chlorobenzhydrol • HCl]	15.0 mg.
diphenylpyraline HCl (1-methyl-4-piperidyl-benzhydryl ether • HCl)	1.0 mg.
phenylephrine HCl	5.0 mg.
glyceryl guaiacolate	100.0 mg.
alcohol	6%

ULOGESIC® — Each tablet contains:

chlophedianol HCl* [alpha-(2-dimethylaminoethyl)-o-chlorobenzhydrol • HCl]	7.5 mg.
diphenylpyraline HCl (1-methyl-4-piperidyl-benzhydryl ether • HCl)	0.5 mg.
phenylephrine HCl	2.5 mg.
glyceryl guaiacolate	25.0 mg.
acetaminophen	162.5 mg.

*Patents pending

INDICATIONS: For acute cough associated with:

Upper Respiratory Infections		Bronchitis
Common Cold	Pertussis	Tracheitis
Influenza	Pleurisy	Laryngitis
Pneumonia		Croup

Allergies (Ulominic and Ulogesic)

CONTRAINDICATIONS: Although no contraindications for ULOMINIC or ULOGESIC are known, they should be used only for acute cough.

CAUTION: Since ULOMINIC and ULOGESIC contain an antihistaminic agent, drowsiness may occur. As they also contain a sympathomimetic agent, they should be used with caution in coronary artery disease, glaucoma, hypertension, and hyperthyroidism.

SIDE EFFECTS:

ULO
These occur only occasionally and have been mild. Nausea and dizziness have occurred infrequently; vomiting and drowsiness rarely. As with all centrally acting drugs, an infrequent case may develop excitation, hyperirritability and nightmares. The symptoms disappear within a few hours after the drug is discontinued. In three cases (1 adult and 2 children) where the drug was continued in large or even excessive amounts after stimulation was present, hallucinations developed. Upon withdrawal of the medication, the patients recovered rapidly within a few hours.

ULOMINIC and ULOGESIC
Side effects from ULOMINIC or ULOGESIC occur occasionally and are mild. Nausea, dizziness, and dryness of the mouth occur infrequently: vomiting and drowsiness rarely.

DOSAGE:

ULO
Adults: 25 mg. (1 teaspoonful) 3 or 4 times daily as required.
Children: 6 to 12 years of age—12.5 to 25 mg. (½ to 1 teaspoonful) 3 or 4 times daily as required;
2 to 6 years of age—12.5 mg. (½ teaspoonful) 3 or 4 times daily as required.

ULOMINIC
Adults: One teaspoonful (5 cc) four times daily.
Children: 6 to 12 years—½ teaspoonful (2.5 cc) 4 times daily.
2 to 6 years—¼ teaspoonful (25 drops) 4 times daily.

ULOGESIC
Adults: Two tablets 4 times daily.
Children: 6 to 12 years—one tablet 4 times daily.

AVAILABILITY:

ULO SYRUP
Bottles 12 oz.

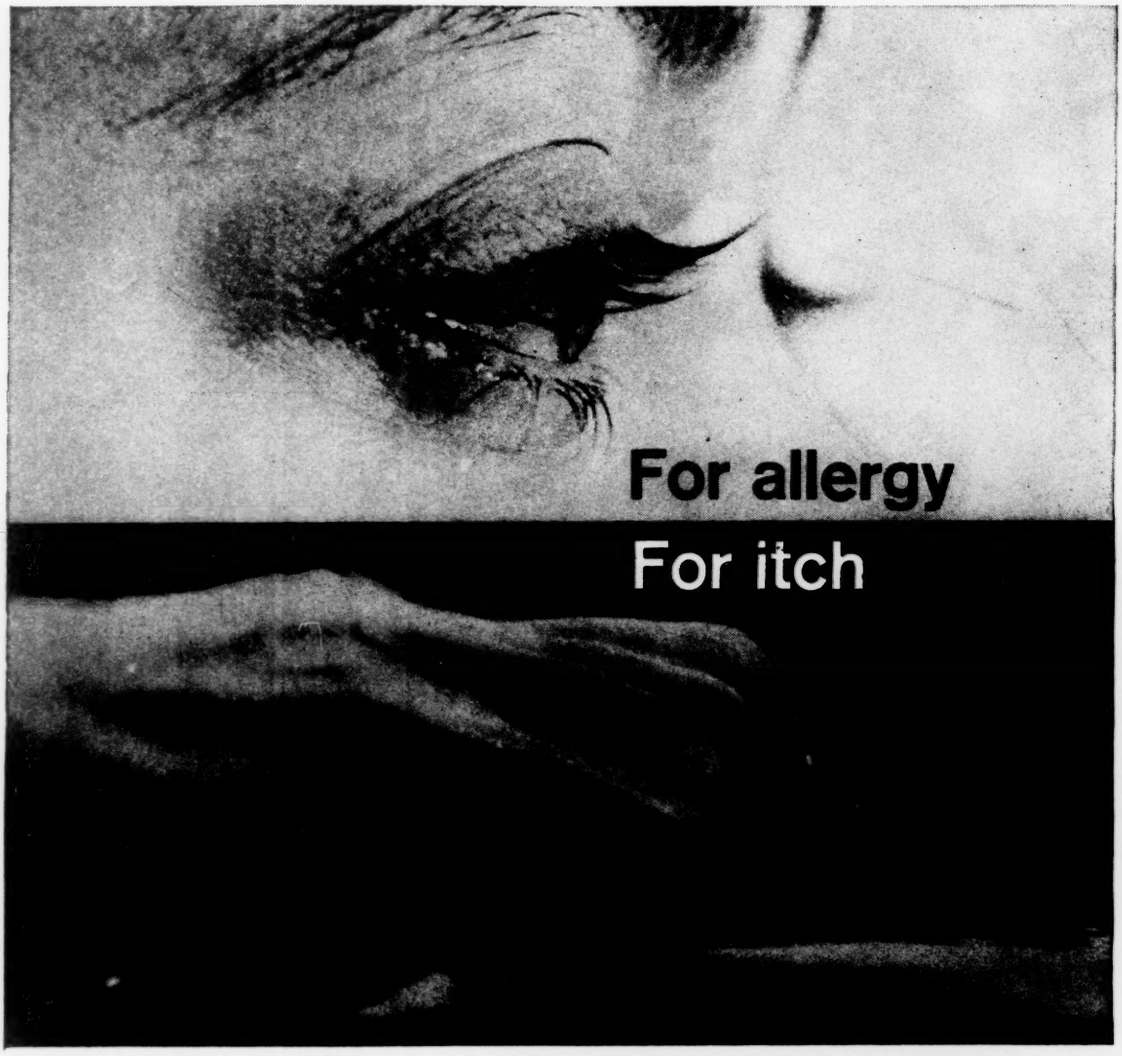
ULOMINIC SYRUP
Bottles 1 pint

ULOGESIC TABLETS
Bottles of 100 tablets.

CAUTION: Federal Law prohibits dispensing without prescription.

Riker

RIKER LABORATORIES, INC., Northridge, California



Everyday practice report:

Following initial clinical investigational work, Forhistal was sent to physicians throughout the country for evaluation as an antiallergic and antipruritic agent in everyday practice. Results in 4026 cases have now been analyzed. In 2260 cases in which a comparison was made, Forhistal was judged better than previous therapy in 7 out of 10 patients. Information about the investigational work done previously is being mailed to you separately and is also available on request.

SUPPLIED: *Tablets*, 1 mg. (pale orange, scored). *Lontabs*, 2.5 mg. (orange). *Syrup* (pink), containing 1 mg. Forhistal maleate per 5-ml. teaspoon. *Pediatric Drops* (pink), containing 0.5 mg. Forhistal maleate per 0.6 ml.

For complete information about Forhistal (including dosage, cautions, and side effects), see Physicians' Desk Reference or write CIBA, Summit, N.J.

FORHISTAL® maleate
(dimethpyrindene maleate CIBA)
LONTABS® (long-acting tablets CIBA)

CIBA
SUMMIT, NEW JERSEY

Forhistal® rated better than previous therapy in 7 cases out of 10

Forhistal Lontabs® for greater convenience, smoother response, prolonged action.

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